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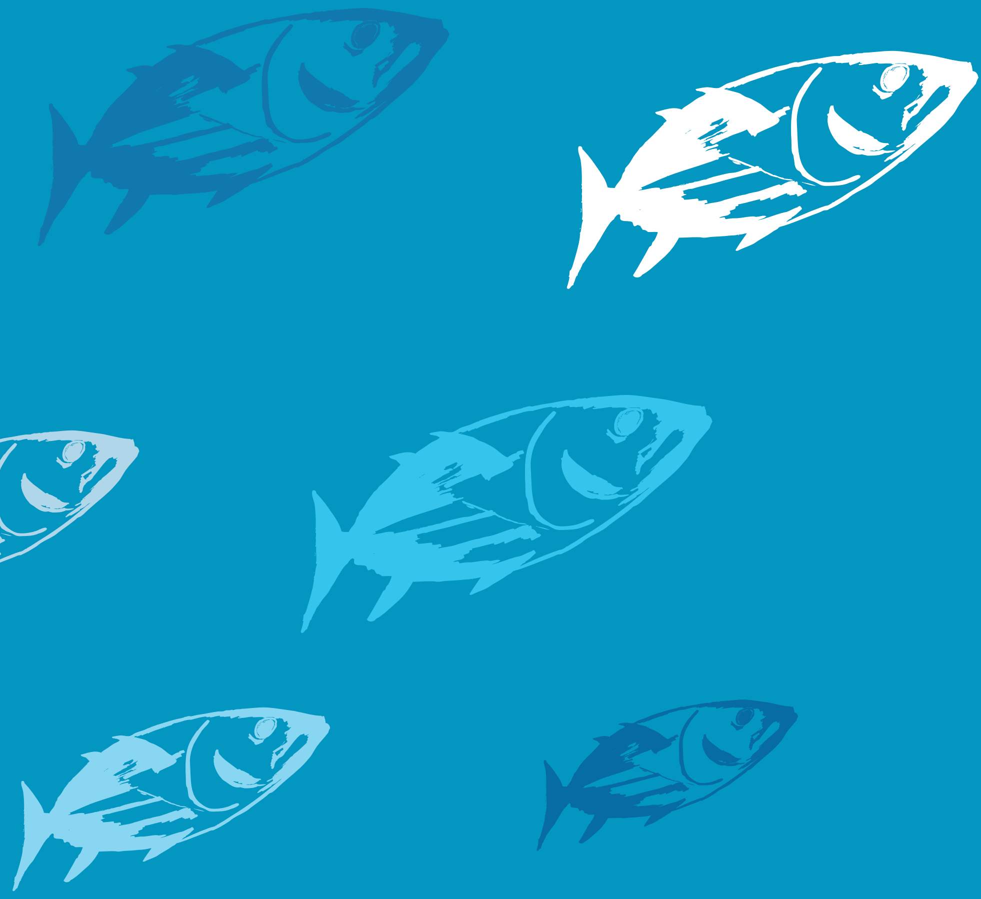
The reproductive biology, condition and feeding ecology of the skipjack, *Katsuwonus pelamis*, in the Western Indian Ocean

Maitane Grande Mendizabal



PhD Thesis
Department of Zoology and Animal Cell Biology
2013

eman ta zabal zazu

Universidad del País Vasco
Euskal Herriko Unibertsitatea

The reproductive biology, condition and feeding ecology of the skipjack, *Katsuwonus pelamis*, in the Western Indian Ocean

Tesi zuzendariak, Hilario Murua eta Nathalie Bodin

Maitane Grande Mendizabal

2013ko Apirilaren 26ª

TESIAREN ZUZENDARIAREN BAIMENA TESIA AURKEZTEKO

Hilario Murua Aurizenea jaunak, 34108169-C I.F.Z. zenbakia duenak "The reproductive biology, condition and feeding ecology of the skipjack; *Katsuwonus pelamis*, in the Western Indian Ocean" izenburua duen doktorego-tesiaren zuzendari naizenak, tesia aurkezteko baimena ematen dut, defendatua izateko baldintzak betetzen dituelako. Maitane Grande Mendizabal doktoregai andreak egin du aipaturiko tesia, AZTI Tecnalia-ko Itsas-Ikerketa sailean.

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Iz.: Hilario Murua Aurizenea

Universidad del País Vasco Euskal Herriko Unibertsitatea

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Nathalie Bodin andreak, pasaporte zenbakia: 07AX12437 eta 060537202886 I.F.Z . zenbakia duenak "The reproductive biology, condition and feeding ecology of the skipjack, *Katsuwonus pelamis*, in the Western Indian Ocean" izenburua duen doktorego-tesiaren zuzendari naizenak, tesia aurkezteko baimena ematen dut, defendatua izateko baldintzak betetzen dituelako. Maitane Grande Mendizabal doktoregai andreak egin du aipaturiko tesia, AZTI Tecnalia-ko Itsas Ikerketa sailean.

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Iz.: .Nathalie Bodin

Universidad del País Vasco
Euskal Herriko Unibertsitatea

TESI-BEGIRALEAREN BAIMENA TESIA AURKEZTEKO

Nik, **Maren Ortiz Zarragoitia** jaunak, tesi-begiralea naizen aldetik,
baimena ematen dut honako tesi hau aurkeztua izateko, horretarako baldintza guztiak biltzen baititu:

Tesia: “The reproductive biology, condition and feeding ecology of the skipjack; *Katsuwonus pelamis*, in the Western Indian Ocean”

Tesia egin den saila: Zoologia eta Animali Zelulen Biologia

Tesiaren egilea: Maitane Grande Mendizabal

Tesiaren zuzendaria: Hilario Murua Aurizenea eta Nathalie Bodin

Leioa (e)n, 2013ko Apirilaren 24 (e)an

TESI-BEGIRALEA

Sin.: Maren Ortiz Zaragoitia

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SAILAREN ADOSTASUNA

Zoologia eta Animalia Zelulen Biologia Saileko Kontseiluak, 2013ko Apirilaren 10eko (e)ko bileran, “The reproductive biology, condition and feeding ecology of the skipjack, *Katsuwonus pelamis*, in the Western Indian Ocean” izenburua duen doktorego-tesia aurkeztearen alde dagoela adierazi du.

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___________________, _______(e)ko ________________aren ______a

O. E. SAILEKO ZUZENDARIA   SAILEKO IDAZKARIA

Iz.: ___________________________   Iz.:_______________________

emman ta zabal zazu

Universidad del País Vasco
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DOKTORE GRADUAREN AKTA
DOKTOREGO TESI DEFENTSAREN AKTA

Doktoregai jn./and.: **Maitane Grande Mendizabal**

TESIAREN IZENBURUA: **The reproductive biology, condition and feeding ecology of the skipjack, *Katsuwonus pelamis*, in the Western Indian Ocean**

UPV/EHUko Doktoregoko Azpibatzordeak goian adierazitako doktorego tesia kalifikatzeko aukeratutako epaimahaiak, eta adierazitako egunean bilduta, behin doktoregaiak defentsa eginda eta egin zaizkion objekzioak edota iradokizunak erantzunda, ___________________ *(Aho batez edo gehiengoaz)* eman du honako kalifikazioa:

GAI edo EZ GAI

Defentsan erabilitako hizkuntzak (hizkuntza bat baino gehiago erabili badira, zehaztu hizkuntza bakoitzean defendatutako atalak edo ehunekoak): ____

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1. mahaikidea, 2. mahaikidea, 3. mahaikidea,

Sin.: Sin.: Sin.:

Dk: ______________________ Dk: _________________________ Dk: _____________________

DOKTOREGAIA,

Sin.: ______________________

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- Grande, M., Murua, H., Zudaire, I., Goñi, N., Submitted. Sex ratio, reproductive timing and batch fecundity of the skipjack in the Western Indian Ocean. J. Fish Biol.

- Grande, M., Murua, H., Pernet, E. J. A., Pernet, F., Zudaire, I., Bodin, N. Submitted. Lipids in female skipjack, *Katsuwonus pelamis*, tuna from the western Indian Ocean: Influence of the reproductive cycle and the fishing environment. Mar. Biol.

- Grande, M., Murua, M., Zudaire, I., Potier, M., Ménard, F., Goñi, N., Bodin, N., Submitted. Link between the diet and reproductive activity of the skipjack, *Katsuwonus pelamis*, in the Western Indian Ocean. Mar. Ecol. Prog. Ser.

Contents

1. Laburpena

Egun, lanpo sabel-marradunaren (*Katsuwonus pelamis*) arrantza, munduko hiru ozeanoetako eremu tropikal eta subtropikaletan burutzen dena, arrantza jarduera ekonomiko garrantzitsuenetarikoa bilakatu da. Horren adibide urtero harrapatzen diren interes komertziala duten atunen 4.2 milioi tonetatik %58a lanpo sabel-marraduna izatea delarik (Majkowski *et al.*, 2011; ISSF, 2012). Indiako Ozeanoan atun espezie honen harrapaketa guztizkoaren %17a (398,000 tona) da, arrantza honek duen garrantziaren argi islatuz (Arrizabalaga *et al.*, 2012; ISSF, 2012). Atun arrantzaren jarduera nagusia, Indiako Ozeanoaren mendebaldean burutzen da. Espezie honek erakuzten duen produktibitate altua dela eta, Indiako Ozeanoaren Atun Batzordeak (Indian Ocean Tuna Commission IOTC ingelesez) burututako azkeneko ebaluazioak ez du lanpo sabel-marradunaren harrapaketak mugatzeko beharrik aurreikusi espeziaren egoera ona dela eta (IOTC, 2012). Hala ere, Indiako Ozeanoaren mendebaldean espezie honi dagokion ugal biologia eta ekologia ikertzen duten lanak ez dira ugariak eta informazioa ez dago eguneratuta, nahiz eta ezinbeztekoa izan arrantza kudeaketa egoki bat gauzatzeko. Hori dela eta, IOTC-k lanpo sabel-marradunaren biologiaren ikerketaren sustapena aldarrikatzen du. Bestalde, inguraketa atun arrantza eta, batez ere, objektu flotatzaileetan bilduriko arraia-sarden arrantza (Fish Agregating Devices “FAD” ingelesez), Indiako Ozeanoan eta mundu mailan etengabe zabaltzen ari da; egun Indiako Ozeanoan ematen diren inguraketa lanpo sabel-marradun arrantzaren harrapaketen %85a izanik (Herrera *et al.*, 2012). Objektu flotatzaileek erruleen elikadura eta egoera fisiologikoan efektu kaltegarriak izan ditzaketela uste da (Fonteneau *et al.,* 2000; Dagorn *et al.*, 2012), ondorioz, erruleen ugal-ahalmena kaltetua izanik. Beraz, atun espezie honek arrantzarekiko duen berreskuratze gaitasuna aztertzeko ezinbestekoa da espeziearen ugalketan eragiten duten faktore biotiko eta abiotikoak ikertzea (Trippel, 1999), espeziearen ugalketaren arrakastan egonik etorkizuneko populazioaren biziraupenaren kako garrantzitsuenetako bat. Hortaz, lanpo sabel-marradunaren ugalketaren ezagutzan aurrerapausoak emateko asmoarekin, doktorego tesi ikerketa honen helburua Ozeano Indikoko lanpo-sabelmarradun populazioren ugal ahalmenaren aldakortasunean eragiten duten ugal-ezaugarriak, eta erruleen ezaugarri fisiko eta fisiologikoek hauengan duten influentzia, aztertzea da.

Ugal-ahalmena aztertu aurretik arrainek ugalkortasuna erregulatzeko duten modua (mugatua edo mugagabea) xehetasunez ikertu behar da, errugulazio motan bait datza arrainen arrautza-ekoizpena balioztatzeko metodologiaren aukerapena (Murua eta

Saborido-Rey, 2003; Murua *et al.*, 2003; Armstrong eta Witthames, 2012). Lanpo sabel-marradunaren ugalketa aztertu duten zenbait lanek, zeharka, honek ugalkortasun mugagabea erakusten duela edierazten dute (Cayré eta Farrugio, 1996; Stéquert eta Ramcharrun, 1995; Stéquert eta Ramcharrun, 1996; Timohina eta Romanov, 1996). Hala ere, ikerketa hauek ez dituzte Hunter *et al.* (1992), Greer Walker *et al.* (1994) eta Murua eta Saborido-Rey (2003) proposatutako irizpideak aztertzen, non ezinbeztekoak diren ugalkortasuna erregulatzekoa modua zehazteko. Beraz, doktorego tesi honen **Lehengo kapituluaren (Chapter I)** helburua lanpo sabel-marradunak ugalkortasun mugagabea duela frogatzea da bibliografian definitatutako irizpideak jarraituz: (a) obozito diametroaren frekuentziaren distribuzioaren jarraitasuna ikuskatu zen; (b) errute garaian zehar obozitoen garapen-estadio bakoitzaren aldakortasun erlatiboa aztertu zen; (c) errute garaian zehar erruteko prest zeuden emeetan obozito bitelatuen (Vtg3) bataz besteko diametroaren aldagarritasuna aztertu zen; (d) azkenik errute garaian zehar atresiaren eragina zenbatetsi zen. Gure helburua lortzeko, emeen gonadak histologia bitartez (n=673) eta irudi analisia erabilita (n=93) analizatu ziren. Batutako informazioa aztertuz lanpo sabel-marradunak obario garapen asinkronikoa, ugalkortasun mugagabea duela eta errule partziala dela ondorioztatu zen. Hau da, errute sasoian zehar arrautzak txanda ezberdinetan jartzen dituela eta arrautzen ekoizpena errute garaian zehar etengabea dela ikustatu zen. Ondorioz, populazioaren ugalkortasuna errute garaia, frekuentzia (txanden arteko tartea) eta txanda bakoitzean ekoiztutako arrautza kopurua erabiliz estimatu beharra dago.

Bestalde, ugal-ahalmenaren ikerkuntzan, populazioaren ugal ahalmenaren aldakortasunean eragiten duten ugal-ezaugarriak aztertzea ezinbestekoa da (Trippel, 1999). Ugalketan, erabilgarria den energia, biziraupena eta hazkuntzaren arteko balantzearen ondorioz geratzen den energia erabiltzen da (Wootton, 1998). Horrela, arrai bakoitzak ez ditu ugal ezugarri berdinak edo ekarri berdina izango populazioaren ugal-ahalmenari dagokionez. Hauek erruleen adinaren, tamainaren eta egoera fisiologikoaren menpe egongo dira, aldakortasun handia izanik denbora eta lekuaren arabera (Marshall *et al.*, 1999; Marshall *et al.*, 2003; Lambert, 2008; Kjesbu, 2009). Nahiz eta ugal-ahamenean eragindako aldaketak zuzenean populazioaren erreklutamentuan eta, beraz, biziraupenean eragina dutela jakin (Rideout and Tomkiewicz, 2011); 90. hamarkada geroztik Indiako Ozeanoan lanpo sabel-marradunaren ugalketa ez da sakonki ikertu (Stéquert eta Ramcharrun, 1995; Stéquert eta Ramcharrun, 1996). Ondorioz, **Bigarren kapituluan (Chapter II)** lanpo sabel-marradunaren ugal-ezaugarriak aztertu eta hauek ezaugarri fisiko eta fisiologikoekin duten erlazioak aurkeztuko dira. Lagindutako arrainen sexua identifikatuz emeen eta arren kopurua parekatuta zegoela ikusi zen. Lagindutako emeen gonadak (n=673) histologia bidez analisatu ziren eta lortutako ugal informazio zehatza baliabide izanik populazioaren heldutasuna eta errute garaiak zehaztu ziren. Emeen lehen heltze sexualaren luzera, $L_{50,}$ 39.9 cm-tan zenbatetsi zen. Orain arte, Stéquert eta Ramcharrun (1996) lana kontutan izanda, IOTC-k lehen heltze sexualaren luzera handiagoa onetsi izan du, 43 cm. Aldaketa honen zergatia erabilitako metodologian datza, non erabilitako histologia metodoak lanpo sabel-marradunaren heltze sexuala ugalketa zikloaren fase goiztiar batean identifikatzea ahalbidetu duen. Badirudi, garapen azkarra eta arrautza ekoizpen handia duten espezie hauetan egokiagoa dela erabili berri den irizpidea (Wright, 2007; Lowerre-Barbieri *et al.,* 2011*a*). Errute sasoiari dagokionez, emaitzen arabera badirudi lanpo sabel-marradunak urte osoan zehar arrautzak erruten dituela, ugal-ahalmena handiagoa ipar-ekialdeko (urtarrila eta otsaila bitartean) eta hego-mendebaldeko (ekaina eta uztaila bitartean) montzoi sasoietan erakutsiz. Hala ere, indize fisiologikoen aldakortasun handia kontutan izanda populazio mailan ugalketa asinkronikoa erakusten duela esan dezakegu. Arrautza-ekoizpena balioztatzeko helburuarekin, txanda bakoitzean jarritako arrautzak metodo grabimetrikoa erabilita zenbatu ziren (n=51). Txanda bakoitzean 0.1 -tik 0.6 milioira edo 140 ± 64 arrautza eme gramo bakoitzeko ekoitz ditzakeela zenbatetsi zen. Arrautza-ekoizpena eta indize gonadosomatikoa ez daude zuzenki eralazionatua arrainaren tamaina edo adinarekin. Badirudi, emeek erruteko gai diren lehen urtean zehar ugal-ahalmen handia erakusten dutela. Hau banako errute garaiaren eta arrainaren egoeraren (indize hepatosomatikoa) menpe egonik.

Esan bezala, arrainen egoera fisiologikoa arrautza ekoizpenarekin positiboki erlazionatua dago (Kjesbu *et al*., 1991; Kjesbu *et al.*, 1998; Marshall *et al*., 1999; Kjesbu, 2009). Hala ere, aldagai bi hauen arteko lotura arrainak ugalketan erabiliko duen gaineratiko energia erabiltzeko aukeratutako estrategiaren menpe dago (Kjesbu, 2009). Bi estrategia nabarmentzen dira. Alde batetik, erreserba energetikoen independente eta elikaduraren bitartez mantentzen den ugalketari “income breeding” deritzo. Bestalde, obozitoak ekoizteko ahalmena emeak aurrez aurretik pilatutako energiaren menpe dagoenean “capital breeding” bezala ezagutzen da, non ekoizpena eta emearen egoera fisiologikoa estuki erlazionatuak dauden kasu honetan (Drent eta Daan, 1980; Stearns, 1989). Orain arte, atun tropikaletan eta beriziki lanpo sabel-marradunean ugalketa garaian zehar ematen den energia dinamika ez da ikertu, azken hau analisatzea ezinbestekoa izanik arrautz ekoizpenean eragin dezaketen faktoreak identifikatzeko. Hortaz **Hirugarren kapituluan (Chapter III)** ugal zikloan zehar lipidoen banaketa deskribatu eta honek ugal-ahalmenean duen influentzia aztertzen da. Geruza fineko kromatografia erabilita, emeen ehun ezberdinen (gonada, gibel eta muskuluan) lipido moten (i.e. TAG, PL, ST, SE-WE, KET eta AMPL) kontzentrazioak balioztatu ziren (n=110). Ugal estrategia energetikoa ezagutzeko, ugalketa zikloan zehar lipidoak gonaden ugal egoera desberdinetan erakusten zituzten aldaketak (bai lipido bai konzentrazio desberdinak) aztertu zen. Horrez gain lipidoen kontzentrazioak eta adierazle fisiologiko, morfologiko (luzeraren) eta arrautz-ekoizpenak zuten erlazioa zenbatetzi zen. Azterlan honen emaitzetatik indize gonadosomatiko, hepatosomatiko, eta gonada eta gibeleko lipidoen bataz besteko kontzentrazioa emeen heldutze prozesuarekin batera handitzen zela balietsi zen, balio altuenak errute sasoian zeuden banakoetan azalduz (I_G=3.1±1.0; I_H=1.8±0.5; lipido totalak gonadan=18.0±0.*1 µg.mg*$^{-1}$*ww*; lipido totalak gibelean= 19.8±0.1 *µg.mg*$^{-1}$*ww*). Indize somatikoak (2.0±0.2) eta muskuluan neurtutako lipidoek (4.8±0.2 µg.mg^{-1}ww*)* ez zuten aldakortasun adierazgarririk erakutzi. Bestalde, gonadan neurtutako erreserbako lipidoak (TAG eta SE-WE) arrautz ekoizpenarekin positiboki erlazionatuta zeudela ikusi zen, gibelean eta muskuluan neurtutakoak ez bezala. Badirudi, espezie honek errute garaia ekiteko aurrez ez duela energi pilatzen . Horrez gain, ez zen ugalkortasuna eta gibeleko eta muskuluko lipido erreserben artean erlaziorik topatu. Beraz, lanpo sabel-marradunak "income breeding" izendatutako energia pilaketa estrategia erakusten duela ondorioztatu dezakegu. Horrela, obozitoen garapena eta ekoizpena errunaldian zehar asimilatutako elikagaiengandik lortutako energiak baldintzatzen duela uste da. Ugal-ezaugarri honek, obario garapen asinkronikoarekin eta ugalkortasun mugagabearekin bat egiten du. Ustez, erreserba energetikoen gabeziak ez du ahalbidetzen ekoiztu beharreko obozitoen zenbatekoa aurreikusten, eta beraz, hauek asinkronikoki garatzen dira elikadurarengatik lortutako energia dela medio (Saborido-Rey *et al*., 2010; Alonso-Fernandez and Saborido-Rey, 2012). Bestalde, objektu flotaitzaileek erruleen egoera fisiologikoan izan zitzaketen ondorio kaltegarriak aztertzeko ahaleginean, arraia-sarda askeetan eta objektu flotatzaile inguruetan arrantzatutako erruteko gai ziren emeetan neurtutako lipidoen kontzentrazioen artean ezberdintasunak aztertu ziren. Arraia-sarda askeetan lagindutako emeen gonada eta gibeletako bataz besteko lipido kontzentrazioa altuago zela ikusi zen. Aurretik gauzatutako zenbait lanek adierazi bezala (Hallier eta Gaertner, 2008; Marsac *et al.*, 2000; Jaquemet *et al.,* 2011; Dagorn *et al.,* 2012; Fonteneau *et al.*, 2013) aurkeztutako emaitza hauek ere objetu flotatzaileen inguruan biltzen diren arrainen egoera kaltetua izaten dela dirudi. Hala ere, laginketa estrategia desegokia eta lan honen helburuen kanpo dauden faktore biologiko eta etologikoak direla eta, ikerketa honek gai horren inguruan ez du ondorio tinkorik plazaratu.

Azkenik, lanpo sabel-marradunaren ugal estrategia kontuan izanik eta elikadurak ugal garaian zehar duen eginkizun garrantzitsua aintzat hartuz, **Laugarren kapituluan (Chapter IV)** elikaduraren eta ugalkortasunaren arteko erlazioak aztertzen dira. Hortarako objektu flotatzaileetan arrantzatutako emeen urdailen edukia (n=365) analisatu, eta gibel eta muskuluen konposizio isotopikoa ($\delta^{13}C$ and $\delta^{15}N$) (n=103) balioztatu ziren, azkeneko hauek besteak beste elikaduraren erreflexu bait dira (Martinez del Rio *et al.*, 2009). Lorturiko emaitzetatik, Potier *et al.* (2001, 2002) eta Jaquemet *et al.* (2011)-ek adierazi bezala, objektu flotatzaileetan arrantzatutako lanpo sabelmarradunak egunean zehar elikatzen ez direla egiaztatu zen, aztertutako urdailetako %77a hutsik bait zeuden. Datu honek objektu flotatzaileetan arrantzatutako emeen lipido kontzentrazio baxuekin bat egiten du. Hala ere, arrainen elikadura estrategia ezagutzeko eta objektuek arrainen nutrizioan duten influentzia behar bezala aztertzeko 24 orduak betetzen dituen laginketa beharrezkoa dela uste dugu, gauzatutako honetan eguerditik aurrerako lortutako laginak ez bait dira ugariak. Bestalde, beste espezie batzutan ikusi den bezala (Wootton and Fletcher, 2009; Bapary *et al.*, 2012), populazioaren ugal ahalmena harrapakinen ugaritasunarekin edo hauen kalitatearekin bat handitzen da (esate baterako *Cubiceps pauciradiatus* agerpenarekin); hau itsas produktibitatea areagotzen duten montzoi sasoiekin (ipar-ekialdeko eta hego-mendebaldko montzoia) edota Mozambikeko kanala gurutzatzen duten eddiekin erlazionatuta egonik (Schott *et al.*, 2002; Schouten *et al.*, 2003). Kokapen eta sasoi ezberdinetan lagindutako emeen gibel eta muskuloan balioztatutako $\delta^{15}N$ neurrietan ikusitako aldakortasunak elikaduran ikusitako diferentziak isladatu ezean, kate-trokikoaren oinarrian ematen diren nitrogeno dinamikaren aldaketen ondorioa dela deritzogu, desnitrifikazio/nitrifikazio gradiente latitudinala dela medio (Ménard *et al.*, 2007; Popp *et al.*, 2007). Bestalde, muskulu eta gibelean baliaoztutako $\delta^{15}N$ neurrien arteko diferentzien aldagarritasuna ($\Delta\delta^{15}N_{M-L}$) ezpeziaren migrazio-lekualdatzearen erantzuna dela dirudi. Azkenik, urdailen analisiekin lortutako datuekin ezin izan ziren ugal zikloan zehar energia beharrekin batera gertatutako kontsumo neurrien aldaketak posibleak aztertu, aurretik atun tropikaletan ikusi bezala (McPherson, 1991; Itano, 2000; Bard *et al*., 2002). Gibel eta muskuluan neurtutako $\delta^{15}N$-a emeen egoera sexualarekin erlazionatuta zegoela egiaztatu zen. Aldaketa hauek elikagaien kontsumoarekin ondorioz sortutakoak izan baino, ugal zikloan zehar ehun hauetan gertatzen diren proteinen metabolismoan aldaketen islada direla onetsi zen (Schmidt *et al.,* 2004). Ustez, bitelogenesia gauzatzen duten emeen energi beharren hazkuntzak (Tyler and Sumpset, 1996; Babin *et al*., 2007), muskuluan gauzatutako proteinen degradazioa eta sintesiaren arteko ratioa areagotuko luke, hau bataz besteko $\delta^{15}N$-aren hazkuntzaren erantzule izanik. Datu hau muskuluko proteinak errute zikloan zehar energia iturri gisa erabiltzen diren seinale izan daiteke. Gibelean aldiz, proteinen sintesi eta pilaketak, eta hortaz, aminoazido konposizio aldaketak eta aipatutako ratioaren txikitzeak bataz besteko $\delta^{15}N$-aren jeitsiera ahalbituko luke (Schmidt *et al.,* 2004; Martinez del Rio *et a*l., 2009).

2. Summary

The skipjack tuna, *Katsuwonus pelamis,* is an economically valuable species worldwide, being in the Indian Ocean responsible mainly for the half of the total tuna catches (IOTC, 2012). The quantitative assessment carried out in the Indian Ocean shows that this species is resilient and not prone to over fishing, due to its high productivity (IOTC, 2012). However, information of the reproductive biology and ecology of the species in the Western Indian Ocean is scarce and has, to a large extent, not been updated. On the other hand, the increasing trend of the fishing upon fish aggregating devices (FADs) over free swimming schools (FSC) has arisen the concern about a possible impact of the FADs fishing in the fish population dynamics (Dagorn *et al*., 2012), which may affect the reproductive potential of the species. Therefore, and in order to provide information in support of the scientific advice, this PhD thesis aims to develop research on the stock reproductive potential (Trippel, 1999) by analysing aspects of reproductive biology, bioenergetics and feeding ecology of skipjack tuna. The information obtained in this PhD thesis would enable updating the existing information on reproductive traits of the skipjack in the Western Indian Ocean and would permit to define the control rules governing variations in the reproductive outputs.

Having knowledge of the reproductive strategy in relation to the fecundity type is necessary before the application of any fecundity estimate or egg production method (Murua and Saborido-Rey, 2003; Murua *et al.,* 2003; Armstrong and Witthames, 2012). However, little is known on the reproductive strategy of this species in relation to the model of fecundity regulation (i.e. indeterminate or determinate fecundity regulation) (Murua *et al*., 2003; Kjesbu, 2009). Previous studies suggested that the skipjack shows indeterminate fecundity (Cayré and Farrugio, 1986; Stéquert and Ramcharrun, 1995; Stéquert and Ramcharrun, 1996; Timohina and Romanov, 1996). Nevertheless, the four main criteria provided by Hunter *et al.* (1992); Greer Walker *et al.* (1994) and Murua and Saborido-Rey (2003) that define the determinacy/indeterminacy of a species have never been tested in skipjack. Therefore, the main objective of the **Chapter I** is to define the oogenesis pattern of the skipjack (*Katsuwonus pelamis*) of the Western Indian Ocean basin in terms of oocyte growth and recruitment style in order to test the hypothesis that the skipjack shows indeterminate fecundity. By means of histology (n=673) and image analysis method (n=93) (Thorsen and Kjesbu, 2001) female ovaries were processed in order to explore the four lines of evidence defined by Hunter *et al.*

(1992), Greer Walker *et al*. (1994) and Murua and Saborido-Rey (2003): (a) oocyte size-frequency distribution; (b) seasonal variation of the relative number and percentage of oocyte stages and (c) diameter of the advanced vitellogenic oocytes in females in the spawning capable phase; and (d) incidence of atresia throughout the spawning season. Results showed that this species had a broad oocyte size frequency distribution with no gap formation between the primary and secondary oocyte growth stages. There was no seasonal variation in the percentage of oocyte stages in ovaries in the spawning capable phase, and the diameter of those oocytes at the most advanced vitellogenic stage remained constant during the sampling period. These facts provided evidence of continuous oocyte recruitment into the standing stock of developing oocytes. Moreover, when reaching the end of the active reproductive period (i.e. February and March) the prevalence of atresia increased. This is a mechanism adopted by fishes with indeterminate fecundity to reabsorb the surplus oocyte production. Thus, we stated that the skipjack in the Western Indian Ocean shows asynchronous oocyte growth and an indeterminate fecundity type.

On the other hand, the assessment of fish stock reproductive potential requires to evaluate fluctuations in reproductive traits (e.g. size at maturity, fecundity and spawning season) and to understand underlying mechanisms (Trippel, 1999). Those traits vary temporally and spatially in response to the environmental fluctuations and maternal physiological condition (Wootton, 1984; Marshall *et al.*, 1999; Marshall *et al.*, 2003). Although there is evidence that changes in reproductive traits can impact on fish recruitment (Rideout and Tomkiewicz, 2011), only few studies focused on the reproductive biology of the skipjack tuna from the Western Indian Ocean, the last works in which fecundity was estimated dating from the 80s and 90s (Stéquert and Ramcharrun, 1995), are available. In light of this issue, by means of detailed reproductive data based on a histological staging system, **Chapter II** provides update information on sex ratio, size at maturity and spawning season. In addition, we evaluated the reproductive capacity of the species based on batch fecundity estimates and investigating, for the first time, the relationships between gonadosomatic I_G and hepatosomatic I_H indexes, Fulton´s condition factor, area and season in fish close to spawning. The overall sex ratio did not differ significantly from the expected 1:1. Females (n = 673) were classified into different reproductive phases and this information was used to determine the size at maturity (L_{50}), the spawning season and the batch fecundity (n = 51). Size at maturity for females was estimated as 39.9 cm. Spawning occurs year round with peaks in the reproductive activity during the monsoon events. Demographic differences were detected in the reproductive timing of the population, common fact among tropical fish (Lowerre-Barbieri *et al.,* 2011*a*). 58.7% of the deviance of I_G in fish close to spawning was explained by I_H, K, fish fork length L_F and spatiotemporal factors. I_G was positively related with I_H and the highest values were observed in fish ranging from 45-50 cm during the North-east monsoon. Similarly, batch fecundity and relative batch fecundity (140 ± 64 eggs g^{-1} of fish) did not increase with fish size and the highest values were observed in intermediate size classes. Therefore, we hypothesized that large investment in gamete production was observed in fish in their first spawning year at the beginning of the individual spawning season.

As mentioned, fish condition can significantly affect fecundity (Kjesbu *et al*., 1991; Marshall *et al*., 1999; Kjesbu *et al*., 1998; Kjesbu, 2009). However, the surplus energy allocation strategy adopted by the species would determine the relationships between maternal condition and reproductive success (Kjesbu, 2009). Two strategies are distinguished: the reproduction based on the concurrent food intake independent of energy storage mechanism is referred to income breeding; and the one dependent of the energy stores is defined as capital breeding (Drent and Daan, 1980; Stearns, 1989). In order to understand energy dynamics during the reproductive cycle and elucidate underlying mechanism controlling skipjack fecundity, **Chapter III** analyzes variations in the lipid composition of somatic and reproductive tissues during the reproductive cycle and relationships with condition indices (I_G, I_H and K) of females (n=110). Relationship between egg production indices (i.e. relative number of developing oocyte *RNDO* and relative batch fecundity F_{Brel}) and lipid composition were also evaluated. Finally, this work aimed to investigate differences of lipid composition between spawning capable females (n=66) caught in different areas and seasons under FADs and in free swimming schools (FSCs). As such, we tried to assess whether the spatiotemporal factor and the aggregation behaviour to FADs impacts on the condition and, hence, reproductive capacity of skipjack. Results showed that immature skipjack presented the lowest gonad and liver total lipid (TL) contents. Significant increases of TL, triacylglycerols (TAG), sterol- and wax-esters (SE-WE) and phospholipids (PL) concentrations were reported in these tissues together with the oocytes' maturation until a maximum was reached in spawning capable females. Lipids content in muscle was low and did not show significant variations during reproductive cycle. According to the observed variability of the tissue lipid composition over skipjack maturation cycle, we hypothesized that fat accumulation in the liver is transitory. Liver might buffer the discontinuous effect of feeding, enabling continuous deposition of yolk into developing oocytes during the spawning season (Bonnet *et al.*, 1998; Rinchard and Kestemont; 2003). As such, the skipjack tuna seems to be oriented towards an income breeding strategy because fat accumulation does not occur as an anticipate response to reproduction. In this sense, skipjack fecundity would be mainly dependent on the foraging success during the reproductive season, rather than being provisioned by energy stores. Reproductive traits are not linked at random (Saborido-Rey *et al.*, 2010) and, thus, this strategy is in line with asynchronous oocyte development and indeterminate fecundity type adopted by the skipjack. The lack of fat stores does not permit to the specimens to "predict" the potential number of eggs to be spawned and, therefore, the oocytes are recruited asynchronously at the expense of surplus energy gained concurrently from the diet (Kjesbu, 2009; Saborido-Rey *et al.*, 2010; Alonso-Fernadez and Saborido-Rey, 2012). Moreover, reserve lipids (SE-WE and TAG) and sterols (ST) in gonads were related with egg production estimates unlike somatic tissues, which is a common fact among the income breeders (Stearns, 1992; Kjesbu, 2009). Finally, gonads and liver of fish in spawning capable phase caught in FSCs in the Mozambique Channel during inter-monsoon showed higher lipid content than those caught under FADs in Seychelles and Somalia during North-east and South-west monsoon. Although these results seems in accordance with previous works suggesting a negative effect of FADs on tuna's condition and trophic ecology (Marsac *et al.*, 2000; Hallier and Gaertner, 2008; Jaquemet *et al.,* 2011; Fonteneau *et al.*, 2013), they do not allow to demonstrate a clear effect of FADs on the lipid composition and thus energetic status of spawning capable females due to an unsuitable temporal and spatial stratification of the sampling.

As suggested along the dissertation, feeding remains as one of the most important factor influencing reproduction of the skipjack tuna. Responding to the income breeding strategy, timing of breeding should be related to periods of high food availability; such as periods of high productivity of North-east and South-west monsoons in the Indian Ocean (Schott *et al.*, 2002). In addition, the energy acquired from the metabolised resources depends on its nutrients composition (i.e. increasing from carbohydrates, proteins and lipids) (Olson and Boggs, 1986; Jobling, 1995), and, thus, reduction on the food quality might reduce reproductive investment in skipjack as previously observed in other species (Kjesbu *et al*., 1991; Brooks *et al*., 1997; Lamber *et al*., 2000; Izquierdo *et al*., 2001). Moreover, skipjack might increase its feeding rate while reproducing in response to increasing energy demands (Jonsson, 1997), as previously reported for other tropical tuna, i.e. yellowfin (McPherson, 1991; Itano, 2000; Bard *et al*., 2002). As such, **Chapter IV** aims to investigate the potential relationship between reproductive timing and feeding behaviour by analyzing the stomach contents (n=365) of females captured under FADs and the isotopic composition (i.e. $\delta^{13}C$ and $\delta^{15}N$) of the liver and muscle (n=103). The number of empty stomach was high (77%), as reported in previous works (Potier *et al.*, 2001, 2002; Jaquemet *et al*, 2011). Skipjack does not seem to feed during daylight hours around FADs which could explain the lower lipid contents observed in these fish compared to those caught in FSCs. However, the tuna sampling was dependent on the purse-seine fishing for which sets after mid-day are uncommon. Hence, due to occasional night foraging in skipjack (Schaefer and Fuller, 2005; Romanov *et al*., 2009) and the increasing foraging in surface predators at twilight (Weimerskirch *et al*., 2004), we recommend to conduct a 24h-sampling in order to evaluate the impact of drifting FADs on fish nutrition. In addition, the reproductive capacity of the population seemed to be enhanced during periods of more abundant or high-energy food source (e.g. fish such as *Cubiceps pauciradiatus*), which could be associated with monsoon events, i.e. North-east and South-west monsoons, or related to mesoscale features (e.g. eddies) in the Mozambique Channel. In contrast, tissue isotopic composition did not reflect the seasonal or spatial changes in the diet. Differences observed in $\delta^{15}N$ values were mainly attributed to differences in nitrogen dynamics at the base of the food web (i.e. latitudinal denitrification/nitrogen fixation gradient). In addition, differences in nitrogen isotopic composition ($\delta^{15}N$) between muscle and liver (i.e. $\Delta\delta^{15}N_{M\text{-}L}$) could respond to migratory habits of the skipjack in the Western Indian Ocean. Finally, the stomach contents data did not enable to evaluate changes in the foraging decisions during the reproductive cycle. Fluctuations, on $\delta^{15}N$ in somatic tissues among reproductive phases gave evidence of differences in protein metabolism during the reproductive cycle rather than reflecting changes in the foraging activity. For skipjack in spawning capable phase, liver $\delta^{15}N$ decreased surely in response to an increase in the efficiency of nitrogen deposition (i.e. ratio between protein assimilation and loss) during vitellogenesis. In contrast, the white muscle of these spawning capable fish showed the highest $\delta^{15}N$ which might indicate higher protein degradation to protein synthesis ratio, attributed to the energetic cost of reproduction (Schmidt *et al.,* 2004). Protein mobilization from the muscle may occur to compensate the high energy requirements of fish undergoing the vitellogenesis (Tyler and Sumpset, 1996; Babin *et al*., 2007).

3. Overview

3.1. Context and relevance of the work

Reproduction is the "axis about which the biology of species revolves" (Meien, 1939; cited in Wootton, 1998). The biological success of an individual is measured by the number of offspring that an individual produces in its lifetime (Wootton, 1998). If the fish is observed as and input-output system (Fig. 3.1), the main limiting factors of offspring production are the external energy supplied from the diet, the rate at which this is assimilated, and the trade-off between maintenance, growth and reproductive functions. Maintenance is the preferential energy drain that will allow sustaining the standard and routing metabolism. It will change as the fish grows and after each reproductive episode. Growth reduces the risk of being eaten, increases feeding opportunities, allows the onset of the maturation, and permits affording the high energy cost of reproduction. Finally, when the sexual maturation is attained energy cost of reproduction is high and the energy is invested in the development and production of gametes, reproductive behaviour (e.g. migrations to breeding site, preparation or defence of the spawning site, courtship) or the development of secondary characteristics (e.g. breeding colours, morphological traits). This energy drain would reduce the somatic growth (Roff, 1983; Wootton, 1998; Saborido-Rey and Kjesbu, 2005).

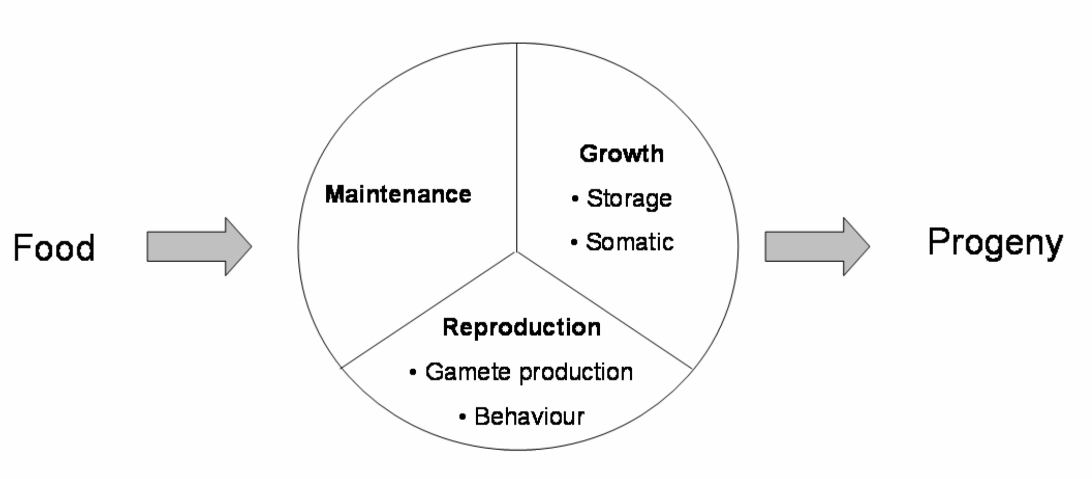

Fig. 3.1. A fish as an input-out system (*adapted from Wootton, 1998*)

In order to succeed in changeable environments, species adopt different strategies that establish the control rules in energy allocation (maintenance-growth-reproduction) to maximize the production of reproductively active offspring in relation to the available energy and parental life expectancy in a given environment (Wootton, 1984; Saborido-

Rey and Kjesbu, 2005; Saborido-Rey, *et al*., 2010). The choice of the suit of rules allows to maintain the species in the forthcoming generations (Wootton, 1984) and determines the relationship between the organism and its environment, and hence the adaptative response of the population to external changes, e.g. fishing (Wootton, 1984; King and McFarlene, 2003; Saborido-Rey, *et al*., 2010). Reproductive strategy is the overall pattern of reproduction common to an individual species and results for a combination of inflexible (e.g. oocyte recruitment style) and flexible traits (e.g. fecundity, maturity ogive, reproductive season) that results from the evolutionary history of the gene pool of which the fish is member (Wootton, 1998). In combination with other life history attributes (e.g. growth and longevity), it results on life-history strategies, e.g. opportunistic, equilibrium and periodic (Winemiller and Rose, 1992; Wootton, 1998).

In a tactical respond to the changeable environment some reproductive traits (e.g. fecundity, maturity ogive, reproductive season) may exhibit an adaptative behaviour which respond to physiological trade-off between reproduction and growth, current reproduction and future reproduction, reproduction and condition, and number and quality of offspring (Stearns, 1992)**.** Several studies demonstrated that fish fecundity is positively related with fish size and spawning experience. Bigger and older fish spawn more and larger eggs which hatch into larger and more viable larvae with major survivorship (Solemdal, 1997; Marteinsdottir and Steinarsson, 1998; Berkeley *et al.*, 2004). In addition, the spawning period is longer for large fish (Hutching and Myers, 1993), and the spawning frequency higher in batch spawner species (Schaefer, 2001*c*). On the other hand, better fed females mature earlier and devote more energy to egg production (Marshall *et al*., 1998; Lambert *et al*., 2000; Lambert *et al*., 2003), while in poor feeding conditions, the oocyte growth rate is reduced (Fletcher and Wootton, 1995) and consequently timing of the spawning might be disrupted (Yoneda and Wright, 2005; Rideout *et al.,* 2005). In such situations, fecundity starts to fall from a critical energy point (Kjesbu, 2009) and fish can skip spawning when energetic reserves are too low (Rideout *et al*., 2000; 2005; Rideout and Tomkiewicz, 2011). In order to fully understand the relative importance of the compensatory mechanism operating at different points of the reproductive cycle, the reproductive strategy adopted by the species has first to be defined (Murua and Saborido-Rey, 2003). These fundamental characteristics would determine the methodological approach to be used in the evaluation of fish reproduction (i.e. main output of reproductive strategies). For instance, the oocyte recruitment strategy adopted by the species (determinate vs. indeterminated) has great importance for the assessment of fecundity (Murua and Saborido-Rey, 2003; Murua *et al.,* 2003) or the choice of egg production methods (Murua *et al*., 2010; Armstrong and Witthames, 2012; Bernal *et al.*, 2012).

While environmental factors, such as temperature and photoperiod, cause medium and long term changes in fish biological traits (e.g. growth, maturity, fecundity, egg quality) (Brooks *et al*., 1997; Lambert *et al*., 2003), fishing can produce rapid alterations (Saborido-Rey and Kjesbu, 2005). Fishery often creates preference to the larger fish inducing change in the length and age structure of the population. As a result of the compensatory mechanism, fish mature earlier and at smaller size (Rochet, 2009). In this sense, the contribution of the older fish to the population productivity is reduced affecting negatively the reproductive potential of the population (Trippel *et al*., 1997). As mentioned the plasticity of life history and reproductive traits allows the stocks to survive to fluctuating environments. However, depending on the strategy adopted by the species, the flexibility of these traits would be insufficient to compensate the increasing mortality (Winemiller and Rose, 1992; Fromentin and Fonteneau, 2001; King and McFarlane, 2003). In light of this issue, and willing to determine in a predictable way how the fishing and environmental factors influence the number of offspring produced by a fish in its lifetime, research studies have been directed to explore fish reproductive strategy, fecundity variability, maternal characteristics, egg viability and larval survival.

Fisheries management most often relies on spawner per recruit models (Ricker, 1954; Beverton and Holt, 1957). In such cases, Spawning Stock Biomass (SSB) is used as a proxy of the Total Egg Production (TEP). The assumed proportionality depends on the proportion of females and the relative fecundity (i.e. number of eggs per unit of female body weight), which are both considered to be constant through time (Marshall, 2009). In this sense, a given weight of mature fish is thought to have the same likelihood of generating the same level of viable eggs. However as cited above this assumption is not generally valid, and thus, management advice based on SSB move away from getting its objective (i.e. the conservation of sufficient reproductive potential of a stock to allow its sustainable exploitation) (Marshall, 2009; Morgan *et al.,* 2009). Consequently, a new term was proposed by Trippel (1999) as an alternative to SSB referred as stock reproductive potential (SRP) which includes parental factors that influence the early life stages related to the recruitment process (quadrant 2-4 in Fig. 3.2). In this sense, scientists no longer view the spawning population as a single mass, and instead, it is partitioned into its different components.

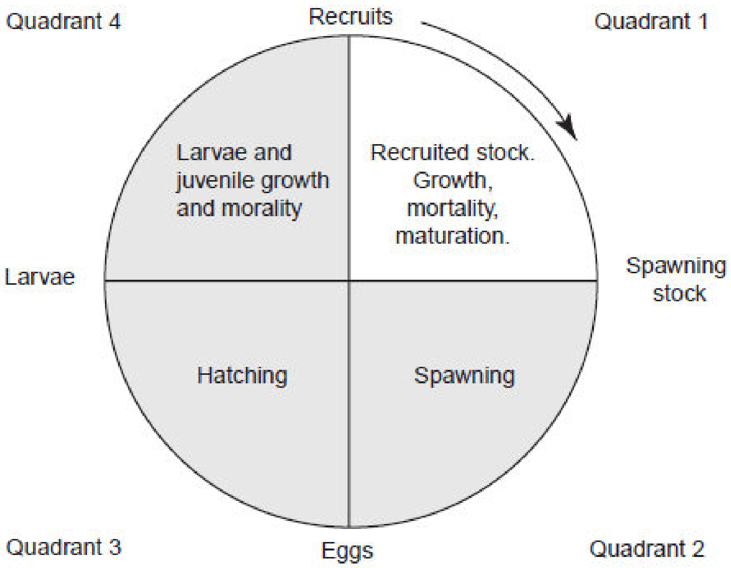

Fig. 3.2. The figure represents factors traditionally included in the population assessment advice. It is generally restricted to the unshaded area (quadrant 1: growth mortality and maturation), while it does not include subsequent life history stages: spawning, hatching and larvae and juvenile growth and mortality (quadrant 2, 3 and 4) (*from Tripper, 1999*)

Despite the growing literature and general acknowledgement of the key importance of accounting for life-history strategies and maternal attributed into fisheries management, little information is available for tuna stocks (Fromentin and Fonteneau, 2000; Juan-Jordá *et al.,* 2012). Therefore the reproductive potential of tuna stocks is not considered into management protocols (IOTC, 2012), while it is indispensable for setting sustainable levels of fishing capacity. Raw data collection and monitoring of reproductive traits and laboratory experiments would help in understanding the basic mechanism regulating the fish fecundity and quantifying the possible substantial contributions of the maternal and environmental factors as well as possible fishing effects on tuna egg production. At the same time, the efforts made on collecting raw data would enable developing alternative, simple and accurate methods for reproductive potential measurements, such as predictors based on condition factors. For example, the development of alternative indices (e.g. hepatosomatic index) of the total number of eggs produced by the stock (e.g. cod) resulted in explaining a higher amount of recruitment variation than did SSB (Marshall *et al.*, 1998; Marshall *et al.*, 2003) and was thus considered as a better proxy of the reproductive potential. However, the validity of the possible fecundity indicators (e.g. condition indexes) depends on the energy allocation strategy adopted by the fish (capital vs. income breeder). While energy reserves are meaningful predictors of egg production for capital breeders, response to diet changes could instantaneous affect gonad development and egg quality in income breeders (Brooks *et al.*, 1997; Izquierdo *et al.*, 2001; Kjesbu, 2009).

The new metholodology developed would enable the routine monitoring of fish stock productivity (Lambert *et al.*, 2003; Marshall *et al.*, 1999; Marshall *et al.*, 2003). Therefore, outputs from this work may provide the basis for the development of biological indicators used for the scientific advice and contribute to the sustainable management of the skipjack fishery.

3.2. Working hypothesis

The working hypothesis can be made up of activities involving the "technique and organs of operation" (Dewey, 1938; cited in Shields and Tajalli, 2006). It is a provisional statement that works as a guide and leads to advance in the investigation (Shields and Tajalli, 2006). Theory shows beforehand where the data are to be fitted. It connects the problem organization, data collection and interpretation. Therefore, it is a useful tool to structure the hyphothesis (Deward, 1938). The experience during the research work provides a tool to adapt to constantly changing framework and guide the inquiry. As such, information in the literature on fish reproductive biology and the experience acquired by the author during the research process help to connect the questions considered to observed data in order to test the following working hypothesis:

Evaluating the reproductive potential of fish stock requires information on reproductive traits and to understand underlying mechanisms that relate those traits with the environment (Tripple, 1999; Lambert et al., 2003; Marshall et al., 2003). To assess the reproductive strategy (i.e. oocyte recruitment and energy allocation) and *traits (i.e. size at maturity, spawning season, fecundity), as well as the links between the maternal attributes (i.e. energetic status and size), the reproductive traits and environmental factors (i.e. food availability) will contribute to the estimation of the stock reproductive potential of the Western Indian Ocean skipjack tuna (Katsuwonus pelamis) stock.*

3.3. Objectives

The present work aims to define the control rules governing variations in the reproductive outputs of the skipjack tuna *Katsuwonus pelamis* in the Western Indian Ocean. This information would enable to update the existing information on reproductive traits and to understand the relationships between reproductive parameters, maternal attributes and environmental conditions. Hence, four specific objectives have been adressed:

- Describe the reproductive strategy of the skipjack in the Western Indian Ocean in terms of oocyte growth and recruitment pattern in order to test the hypothesis that the skipjack shows indeterminate fecundity.
- Assess the reproductive traits of the skipjack in the Western Indian Ocean:
    - Evaluation of the maturity ogive (L_{50}) and spawning season based on histological staging system.
    - Determination of the batch fecundity and evaluation of the ovary growth in spawning females.
- Describe the patterns of lipid accumulation in somatic and reproductive tissues during the reproductive cycle, and the relationship with fish egg production, and asses the effects of the fishing environment (i.e. season, area and school type) on the lipid content in the skipjack of the Western Indian Ocean.
- Investigate the potential relationship between the skipjack reproductive timing and feeding behaviour throught stomach content and stable isotope (i.e. $\delta^{13}C$ and $\delta^{15}N$) analyses.

3.4. Structure of the thesis

The PhD dissertation is arranged as follows:

- It starts with the **Introduction** section. The purpose of this section is to introduce the species to the reader. It provides an up-to-date overview of the biology of the skipjack tuna. Further, worldwide and Indian Ocean fisheries are reviewed and the main concerns related to the exploitation of this tuna stock are described.

- Subsequently, the **Study Area** is defined. This section describes the general circulation and climate variability of the Indian Ocean. The climate is mainly dominated by seasonally reversing monsoon wind system, and thus, major efforts are made in describing the variability on the general oceanography and ocean productivity during these events.

  In order to achieve the objectives defined above the following text has been structured in four chapters. Each of the chapters starts with an introduction which provides an overview of the issue and the specific objectives of the chapter, followed by the methodology applied, results obtained, discussion and conclussions of the main findings.

- **Chapter I*:*** It explores the reproductive strategy of the Western Indian Ocean skipjack in terms of stage-specific and seasonal variability of oocyte size-frequency distribution, seasonal variability of the occurrence of different oocyte stages in spawning capable females, seasonal variation in the mean diameter of the oocytes in the tertiary vitellogenesis stage in spawning capable individuals; and the incidence of atresia through the spawning season. Results allowed to test the hypothesis that the skipjack shows indeterminate fecundity which is a necessary step to choose the appropriate methodology for the estimation of the reproductive potential of the stock.

- **Chapter II**: This section focuses on the assessment of various reproductive traits of the skipjack tuna based on histological staging system and condition indices (Fulton´s condition factor K, hepatosomatic I_{H}, and gonadosomatic index I_{G}), such as the sexual maturity (L_{50}) and spawning season. In addition, the reproductive capacity of the species and its relationships with maternal attributes (i.e. size and condition), and spatiotemporal factors are evaluated.

- **Chapter III**: It presents the lipid composition of somatic and reproductive tissues and analyzes for the first time changes on lipid content over the reproductive cycle of the skipjack in order to evaluate its energy allocation strategy. In addition it explores the relationships between tissue lipid composition, fish size, condition indices (i.e. I_{G}, I_{H} and K) and egg production indices (i.e. relative batch fecundity F_{Brel} and relative number of developing oocytes *RNDO*). Finally, the effects of the fishing environment and mode on skipjack lipid composition are investigated.

- **Chapter IV**: The potential relationships of the skipjack reproductive activity and its feeding behaviour is investigated in this section, by means of the stomach content analysis and isotopic composition (i.e. $\delta^{13}C$ and $\delta^{15}N$) of the liver and white muscle of fish caught in different spatiotemporal strata.

- Then, the **General Discussion** section provides the major findings of the research work developed during the PhD thesis which are discussed as a whole.

- The dissertation ends with the **Conclusions**. This section enumerates the main conclusions drawn from the General Discussion. In addition, the answer to the working hypothesis, i.e. **Thesis,** is given.

4. Introduction

4.1.Taxonomic classification and short description

The skipjack tuna, *Katsuwonus pelamis* (Linnaeus, 1758), is classified into the Scombridae family. Overall, 15 genera and about 50 species form this family (Fig. 4.1) including mackerels, Spanish mackerels, bonitos and tunas which are distributed throughout tropical and temperate waters of the world (Collette *et al.*, 2001).

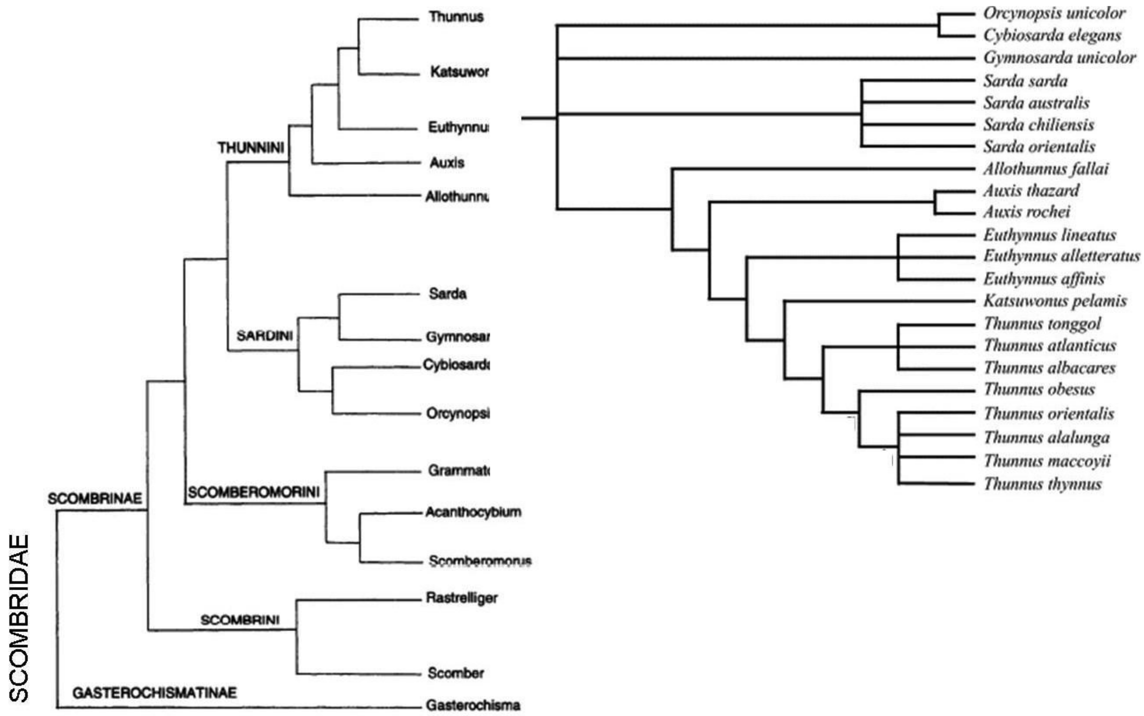

Fig. 4.1. Morphological tree of the family Scombridae based on morphological characters (left side: *from Collette et al., 2001*) and phylogenetic relationships for Thunnini (tunas) of the family Scombridae (right side: *from Bernal et al., 2001*)

Based on morphological studies two subfamilies are recognized: Gasterochismatinae and Scombrinae. The latter is split in four tribes: Scombrini (the more primitive mackerels), Scomberomorini (Spanish mackerels), Sardini (bonitos) and Thunnini (tunas) (Collette *et al.*, 2001; Graham and Dickson, 2004). Five genera are included in Thunnini and four of them (i.e. Thunnus, Katsuwonus, Euthynnus and Auxis) are unique among bony fish. The members of these genera have a counter-current heat exchanger system of retia mirabila in the circulatory system which allows the fish to maintain body tissue temperature warmer than the surrounding waters (Collette *et al.*, 2001; Korsmeyer and Dewar, 2001). This characteristic enables to expand the thermal niche and elevate the aerobic capacity of muscle (Korsmeyer and

Deward, 2001). *Katsuwonus pelamis* is the single species in the Katsuwonus genera. The name *pelamis* was established by Linnaeus in 1758 and gained in acceptance between 1890 and 1935 (Masumoto *et al*., 1984). In English is also known as “oceanic bonito”, ”white bonito”, “striped bonito”, “artic bonito”, “bonito”, “watermelon” or “skippy” (Matsumoto *et al*., 1984). The maximum reported length (i.e. fork length) is of 95 cm (Jones and Silas, 1963). The body is fusiform, elongate and rounded. The dorsal surface is black dark purplish blue and the abdomen is silvery with four to six black longitudinal stripes (Fig. 4.2) (Collette and Nauen, 1983; Matsumoto *et al*., 1984). The body is scaleless except for the corselet and lateral line. The two dorsal fins are separated by a small inter space (not larger than eye), the first with 14 to 16 spines and the second followed by 7 to 9 finlets. The pectoral fins are short and triangular. Gilrakers are numerous (53-63) on the first gill arch. It has more vertebrae than Thunnus (41 compared to 39). It lacks of swim bladder (Collette and Nauen, 1983; Collette *et al*., 2001).

Fig. 4.2. Skipjack tuna (*Katsuwonus pelamis*) (*from www.fischgrosshandel-ahlen.de/Kleine-Fischkunde*)

4.2. Distribution, stock structure, habitat and physiological requirements

Skipjack occurs within the 15° C or warmer isotherm of the world oceans, except for the Black Sea (Fig. 4.3). Specimens have been found as far north as 55º in the Eastern Atlantic and as far south as 45º in the Western Indian Ocean (Colette and Nauen, 1983; Matsumoto *et al.*, 1984).

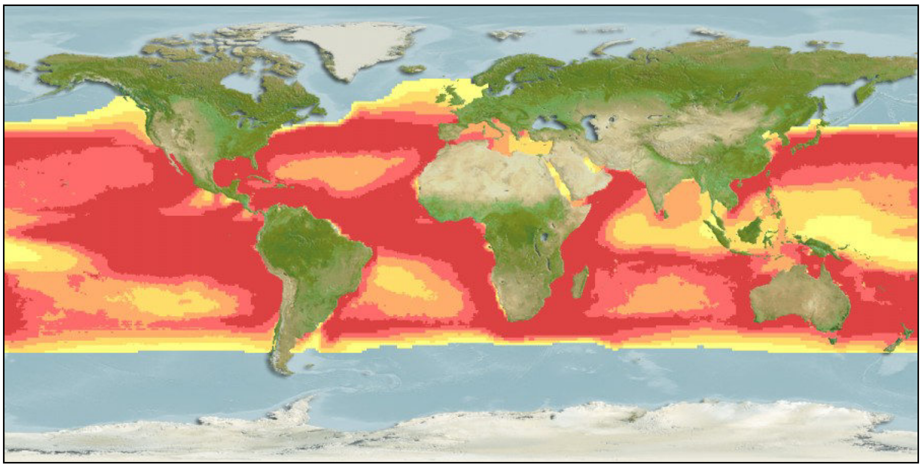

Fig. 4.3. Worldwide geographical distribution of the *Katsuwonus pelamis* (*from www.fishbase.com*)

Studies on population structure on genetic basis are not numerous. High degree of genetic similarity between fish from the Atlantic and Pacific Ocean was observed by Graves and Dizon (1986) and Ely *et al.* (2005) which was attributed to sufficient inter-oceanic migrations and/or large effective population size and lack of discrete spawning areas which increases the possibility of gene flow. In contrast, an earlier study (Fujino *et al.*, 1981) demonstrated the existence of at least four subpopulations (i.e. the Indian, Atlantic, Western Pacific, and Central-eastern Pacific subpopulations), and suggested that the skipjack tuna now inhabiting the world's major oceans first appeared in the Indian Ocean to then emigrate to other oceans. Menezes *et al.* (2006) also observed significant heterogeneity in the distribution of haplotypes between the Indian and Pacific subpopulations. In this context, skipjack tuna are currently managed as single stock units in the Indian Ocean. However, a recent work exploring skipjack in the North-western Indian Ocean (Dammannagoda *et al.*, 2011) provides evidence of the coexistence of two stocks. Two genetic groups were identified in waters surrounding Sri Lanka which were likely to represent individuals from discrete breeding grounds. Menezes *et al.* (2012) also observed the occurrence of four genetically differentiated groups across the coastal waters of India with no clear pattern of geographic locations. Therefore, stock structure in the Indian Ocean could be more complex than thought.

Skipjack vertical and horizontal distribution is limited by the dissolved oxygen and temperature (Barkley *et al.*, 1978; Sund *et al.*, 1981). Lacking of swimblader, tunas, and skipjack in particular, swim continuously to generate hydrodynamic lift and prevent from sinking. High aerobic scope (i.e. which determines the extent to which multiple functions can occur simultaneously) allows for continuous swimming and supplying oxygen to satiate other metabolic demands that are particularly high in skipjack (Korsmeyer *et al*., 1996; Kormeyer and Deward, 2001). As such, skipjack requires waters with unusually high concentrations of dissolved oxygen. Experimental data confirmed that the skipjack is stressed at concentrations below 2.8 ml/l, and 3 to 3.5 ml/l would be required for long term survival (Barkley *et al*., 1978; Sund *et al*., 1981; Wild and Hampton, 1994). On the other hand, temperatures for skipjack occurrence are between about 15 and 30ºC (Barkley *et al*., 1978). Experiments on captive fish resulted on a minimum and maximum temperature tolerance of 15 and 34ºC, respectively (Dizon *et al*., 1977). However, the upper limit tolerated by the species varies with size (Neill *et al*., 1976; Barkley *et al*., 1978). Higher oxygen requirements of large specimens results from larger amount of metabolic heat production of the red muscle, and could lead to dangerously overheat it in warm water regions (Barkley *et al*., 1978; Wild and Hampton, 1994). In this context, dissolved oxygen limits the bathymetric range of skipjack and confines the fish to shallow layers in some tropical regions (Sund *et al*., 1981), while temperature of some of these shallow layers are not suitable for large specimens. Therefore, large specimens are confined to upper thermocline whenever the dissolved oxygen concentration is suitable (Barkley *et al*., 1978).

Other environmental factors limiting the skipjack distribution are the water transparency, prey abundance, climatic oscillations, surface currents and hydrographical features (Sund *et al*., 1981; Matsumoto *et al*., 1984). As visual feeders, skipjack and tunas in general avoid turbid waters in order to enhance their hunting efficiency, while extremely clear waters would limit predation due to the low food abundance (Sund *et al*., 1981). In addition, climatic oscillations (e.g. El Niño Southern Oscillation, Indian Ocean Dipole) (Marsac and LeBlanc, 2000), surface currents (e.g. Kuroshio current) and hydrographic features (e.g. oceanic fronts, mesoescale eddies) (Nihira, 1996; Kirby *et al.,* 2000; Sugimoto and Tameishi, 1992; Ramos *et al*., 1996; Mugo *et al*., 2010; Tew Kai *et al*., 2010) affect the distribution of their prey, water temperature and thermocline topography, and hence, direct or indirectly affect the skipjack distribution.

4.3. Skipjack Fishery

Tunas are important socio-economic resources as well as a significant source of protein for the society. They are subjected to intensive international trade for canning and *sashimi* (i.e. raw tuna highly appreciate by Japanese market) (Majkowski *et al*., 2011). The share of major commercial tunas (i.e. *Thunnus thynnus* or bluefin*, Thunnus maccoyii* or southern bluefin*, Thunnus orientalis* or pacific bluefin, *Thunnus alalunga* or albacore*, Thunnus albacares* or yellowfin*, Thunnus obesus* or bigeye, and *Katsuwonus pelamis* or skipjack) was about 8% of total fish exports in 2010 (FAO, 2012). As a result of the increasing demand for canned tuna, the industrialized fishery started in the 50s with a progressive and continous increase of catches until recent days (Fig. 4.4) mainly due to the expansion of the tropical tuna fishery (i.e. yellowfin, bigeye and skipjack) (Majkowski *et al*., 2011; Arrizabalaga *et al*., 2012). In 2011, the catch of major commercial tuna was 4.2 million tonnes, from which 58% was skipjack mostly for canning (Majkowski *et al.*, 2011; ISSF, 2012).

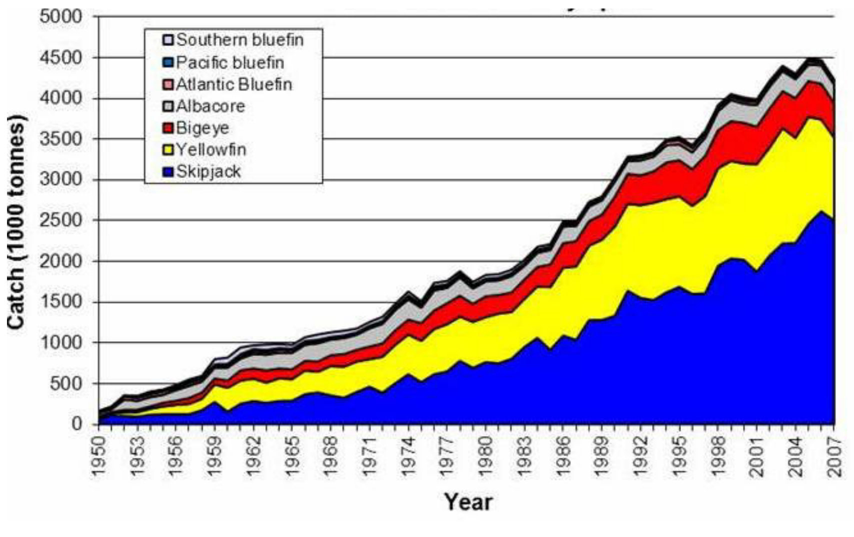

Fig. 4.4. Worlwide catches of the major commericial tunas from the 50st to 2007 (*from Arrizabalaga et al., 2012*).

For management purposes there are 5 commercial skipjack tuna stocks. While a single stock is considered in the Indian Ocean, two stocks are considered in both the Pacific and Atlantic Oceans (the eastern and western stocks). Stocks are assessed and managed by international Tuna Regional Fishery Management Organization (RFMOs):

the International Commission for the Conservation of Atlantic Tunas (ICCAT, www.iccat.int), the Indian Ocean Tuna Commission (IOTC, www.iotc.org), the Western and Central Pacific Fisheries Commission (WCPFC, www.wcpfc.int) and the Inter-American Tropical Tuna Commission (IATTC, www.iattc.org).

Skipjack worlwide tuna catch was the highest in 2009 with a total catch of about 2.5 million tonnes (Fig. 4.5). Skipjack catches during 2010 were mainly coming from the Western Central Pacific with about 68% (1,610,578 t) of global catches. The Indian Ocean accounted for about 18% (417,353 t) of the global catches, followed by the Eastern Pacific (150,661 t), Eastern and Western Atlantic Ocean (182,429 t) which contribute together for about 14%.

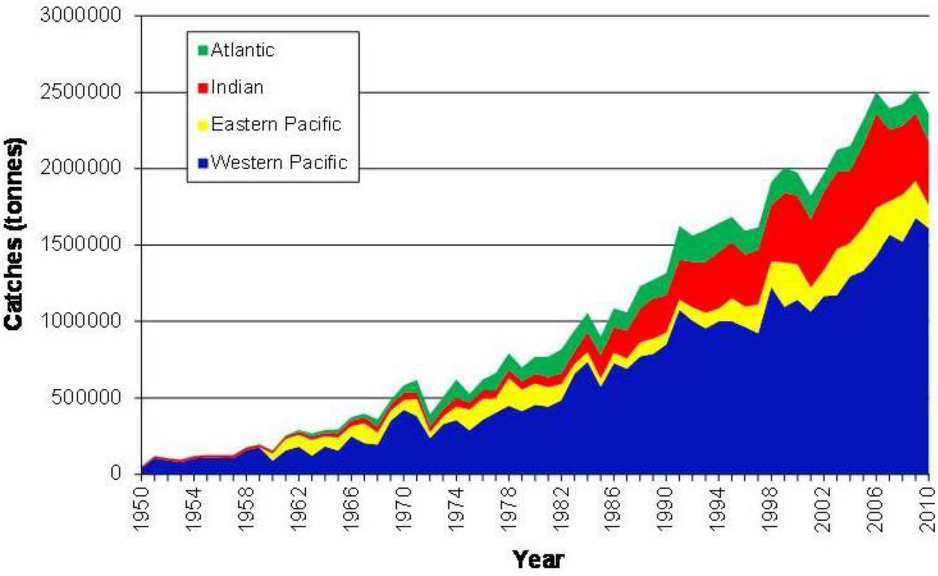

Fig. 4.5. Global trends in catches (tonnes) of skipjack tuna from 1950 to 2011 by Ocean.

Skipjack worldwide fishery is composed by various gears: Purse-seine, pole-and-line, long line and others (Fig. 4.6). At the beginning of the 50s the fishery was mainly composed by pole-and-line vessels. The introduction of seiners in the 60s replaced the pole-and-line fishing mainly in the Eastern and Western Central Pacific Ocean (EPO and WCPO, respectively) and Eastern Atlantic (Arrizabalaga *et al*., 2012). The arrival of the purse-seiners in the Indian Ocean occurred in early 1980s, while in the Western Atlantic even currently the pole-and-line fishing dominates (Arrizabalaga *et al*., 2012). Since 1980s worldwide catches increase steadily due to the expansion of the purse-seine fishing effort. With the introduction of the Fish Aggregating Devices (FADs) in the 90s annual catches increased substantially (Dagorn and Restrepo, 2011).

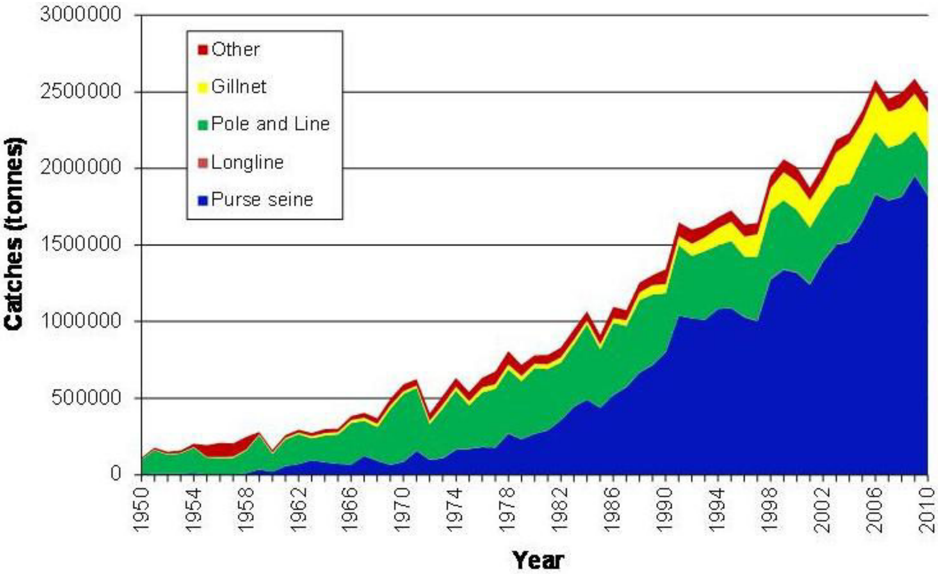

Fig. 4.6. Global trends in catches (tonnes) of skipjack tuna from 1950 to 2011 by fishing gear.

Currently, two major fishing modes are used by purse-seiners: drifting FAD associated schools and free-swimming schools (FSC). FADs correspond to any type of floating object used for increasing tuna catchability due to the aggregative behaviour of tuna underneath the FADs (Fréon and Dagron, 2000; Castro *et al.*, 2002; Dagorn *et al.*, 2012). They can be natural (e.g., logs; Fig. 4.7*a*), or man-made and displayed by fishermen (Fig. 4.7*b*). The later are usually bamboo raft with old netting used to cover the top of the raft and hang down beneath. An electronic buoy is usually attached in the structure which enables the fisherman to localize the object, and some buoys are equipped with echosounder to receive information about the biomass associated underneath (Fonteneau *et al.*, 2000; Dagorn *et al.*, 2012). Therefore, sets on FADs-associated schools have a high level of success (95%) if comparing to FSCs (55%) (Floch *et al.*, 2012). Currently, about 73% of the skipjack worldwide catches are from purse-seine fishing (48% of catches by FADs sets and 25% by FSCs) and 27% by other gears (pole-and-line, longline and others) (Fig. 4.6).

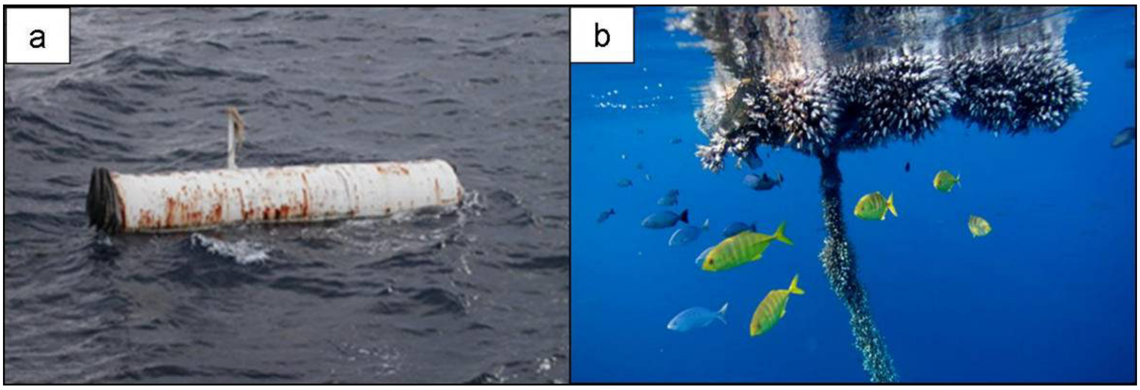

Fig. 4.7. Natural (**a**, from www.nunas.com) and artificial (**b**, from www.alexhoffordphotography.com) Fish Aggregating Devices (FADs).

According to the last assessments, it is unlike that skipjack stocks are overexploited (ISSF, 2012; Arrizabalaga *et al*., 2012). The high productivity and life history characteristics of skipjack tuna suggest that this species is resilient and not easily prone to overfishing. However, the constantly increasing exploitation rate of the skipjack should not exceed the Maximum Sustainable Yield (MSY) and the stocks should be closely monitored (ISSF, 2012; Arrizabalaga *et al*., 2012).

4.3.1. Fishery and state of the stock in the Indian Ocean

In the Indian Ocean catches increased slowly since the 50s (Fig. 4.8*a*) reaching 50,000 t during the 70s due to the pole-and-line and gillnet fishery (Herrera *et al.,* 2012). In the 80s with the arrival of purse-seine vessels catches increased rapidly and skipjack became one of the most important commercial tuna species (Fig. 4.8*c*). Since then, annual total catches has continuously increased to peak in 2006 at over 600,000 t. Currently, skipjack account for 49% of the tropical tuna catches in the Indian Ocean (Herrera *et al*., 2012). 2011 estimates are around 400,000 t which are the lowest recorded since 1998 (Herrera *et al*., 2012).

Contrary to the situation in other oceans, the artisanal fishery component in the Indian Ocean (mainly using pole-and-line, driftnet and hand line) is high, taking about 60% of the total skipjack catches during recent years (2000 to 2008) (Fig. 4.8*a*). Maldivian pole-and-line fishery substantially contributes to the total catches. Since its mechanisation in 1974 and the use of anchored FADs since 1981 catch rate regularly increased (Fig 4.8*a,c,d*). In 2006 maximum catches were recorded (147,391 t), but since that peak, the catches of Maldivian pole-and-line fishery has drop (Herrera *et al.*, 2012). In 2011 it was responsible of the 18% of the skipjack total catches in the Indian Ocean, i.e. 69,032 t (Herrera *et al.*, 2012). Other fishing arts such as gillnet/longline fishery from Sri Lanka, gillnet from India and Indonesia and driftnet from Iran and Pakistan represent 20 to 30% of the total skipjack catches in the India Ocean (Herrera *et al.*, 2012). The catch-and-effort series of these fleets are not available, and therefore, these fisheries are oftenly poorly understood (Herrera *et al.*, 2012)

The purse-seine fleet operating in the Indian Ocean is composed by 34 European and associated flag vessels with a carrying capacity of about 45,000 t, mainly operating in the equatorial area of the Western Indian Ocean (Floch *et al.*, 2012). Due to the increasing fishing power, the number of vessels which moved from the Atlantic Ocean, the use of FADs and the technology associated with them, the catches increased steadily since 1984 with the highest catches recorded in 2002 and 2006 (>240,000 t) (Fig. 4.8*a,c,d*). The catches decreased between 2003 and 2004, probably as a consequence of high purse-seine catch rates on FSCs of yellowfin tuna (Herrera *et al.*, 2012). Purse-seiners contributed to 33% of the skipjack caches (i.e. 132,000 t) during 2011 (Fig. 4.8*a*). Since the 80s, the relative contribution of sets on FAD-associated schools presents a continuous increase (Fig. 4.8a,*c,d*) (Floch *et al.*, 2012). In recent years, the FAD fishing represented 85% of the total skipjack purse-seiner catches (Herrera *et al.*, 2012)

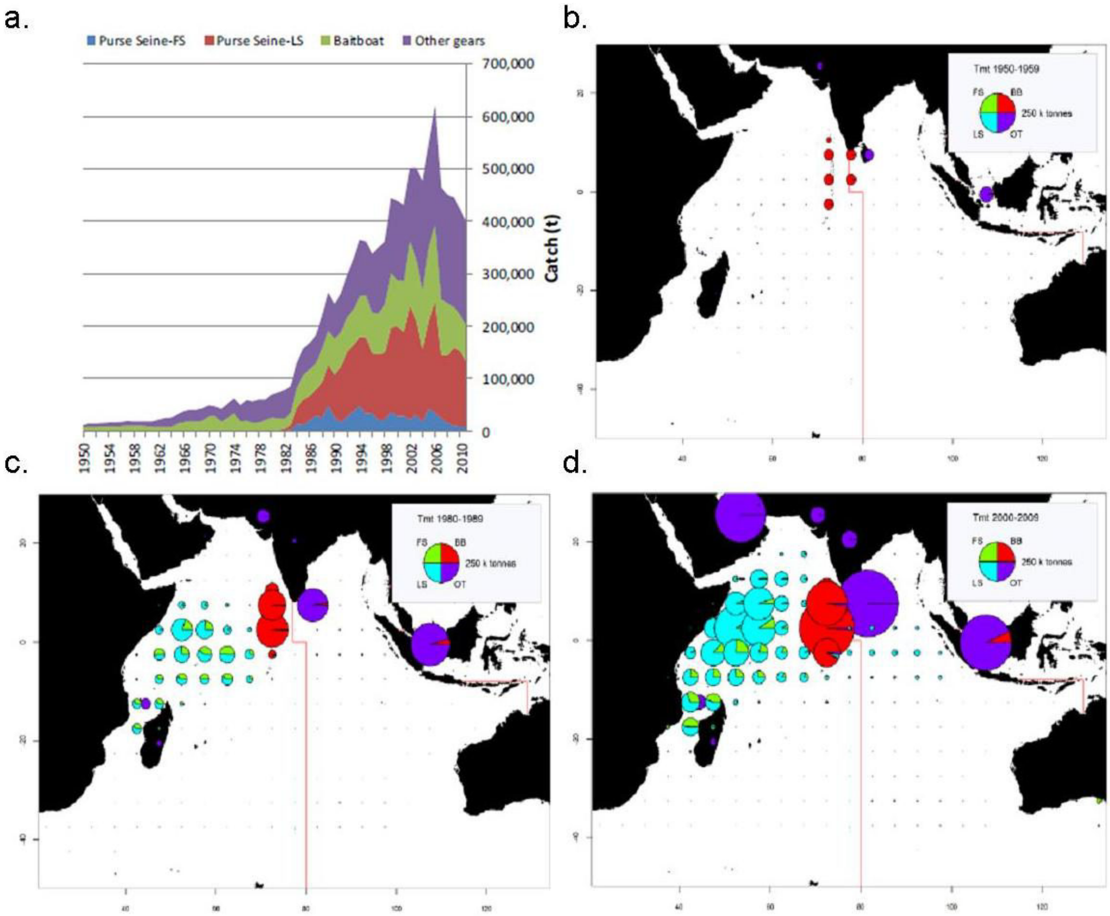

Fig. 4.8. Annual catches of the skipjack tuna by gear from 1950 to 2011 (a, *from IOTC-2012-WPTT14R*). Skipjack tuna time-area catches for the period 1960-1969 (b), 1980-1989 (c) and 2000-2009 (d). Green and blue correspond to purse-seine fishing on Fish Aggregating Devices (FADs) and free swimming schools (FSCs) respectively, red to pole-and-line fishing and purple to others (*from Herrera et al., 2012*)

A general drop in the catches has been observed since 2007, which can be partly explained by the negative effect of the piracy in the Western Indian Ocean region (IOTC, 2012). From 2005 to 2008 the number of European purse-seine fleet decreased by 30%. Fleets from Iran and Pakistan using driftnets and local fleets from Seychelles and Kenya have been also negatively affected (ISSF, 2012). This drop coincided with a similar decline in the Maldivian pole-and-line fishery, which is not related to piracy, but could be caused by the introduction of handlines and the change of the main target species, i.e. from skipjack to large yellowfin (IOTC, 2012*;* Herrera *et al.*, 2012). Also, a noticeable decline of the skipjack mean weight in pole-and-line and purse-seine fishery has been observed since 2007 (IOTC, 2012). The latest stock assessment suggests that skipjack population has high natural mortality, limited selectivity of the youngest spawners and high recruitment compensation (Sharma *et al.*, 2012). Sharma *et al.* (2012) suggest that fluctuations in the catches (e.g. the peak observed in 2006) could partly correspond to high variability in the recruitment, and partly be attributable to fishery. The results of the last assessment suggested that the Indian Ocean skipjack population is not overfished (spawning stock biomass B > spawning stock biomass at MSY, B_{MSY}) and that overfishing is not occurring (catches C < maximum sustainable yield, MSY; and fishing mortality $F < F$ at which MSY can be obtained, F_{MSY}) (IOTC, 2012). In summary, due to the biological characteristics of the species (i.e. fast-growing, early maturation, high fecundity and high natural mortality) the stock in the Indian Ocean seems to be resilience to fishing (IOTC, 2012).

4.3.2. Potential effects of FADs on skipjack populations

The increasing use of FADs has introduced worldwide major changes in the environment of tropical oceans and fishing patterns. In this sense, Fonteneau *et al.* (2000) and Dagorn *et al.* (2012) described the possible effects associated with FAD deployment at sea: alteration of normal movements of tuna, increase of catches in skipjack (the principal target species), reduction in yield per recruit (i.e. in yellowfin and bigeye, from which small specimens co-occur in the catches with skipjack) and increase of bycatch. The potential effect of habitat modification on the behaviour and biology of tunas have been defined under the "ecological trap" hypothesis (Marsac *et al.*, 2000).

Marsac *et al.*, (2000) hypothesized that FAD deployment could alter the natural movements of tuna from biological productive areas where natural logs tend to drift (i.e. converging zones), towards less productive areas where man-made FADs accumulate. If this hypothesis becomes true, FADs may act as an ecological trap affecting negatively tuna growth/condition and increasing mortality while fish remain trapped within high FAD density in unproductive areas. This could be the case of the Indian Ocean as the introduction of FADs has increased floating object density which could also trap tuna (Marsac *et al.,* 2000). However, Fauvel *et al.* (2009) observed that artificial and natural FADs were found in the same areas, and hence, the conditions that could lead to an ecological trap are not observed in this region. However, morphometric studies conducted on skipjack by Marsac *et al.* (2000) and Hallier and Gaertner (2008) showed lower fish condition in associated schools than in free-schools suggesting the negative effects of FADs in associated tunas. One of the possible causes could be the minor feeding success of skipjack while associated with FADs. Numerous works observed that tuna do not feed or feed less while associated with FADs (Ménard *et al.*, 2000*;* Jaquemet *et al.*, 2011). The number of empty stomach is especially high in skipjack (as much as 85%) which seem to be less efficient than juvenile yellowfin seeking out food when associated to the FADs (Jaquemet *et al.*, 2011). However, Robert *et al* (2010) did not attribute the differences in skipjack condition to consequence of the associative behaviour. Skipjack would associate to floating objects after some bad feeding events for schooling, which permits them saving energy while adopting more efficient foraging (Robert *et al.*, 2010). As observed, despite the negative relationship between the FAD associative behaviour and fish condition, the motivations of this association remain unsolved which are indispensable to evaluate the long term effects of FADs use on tuna populations. Dagorn *et al* (2012) claimed that more research is needed to determine why fish are associated with FADs and to explore whether FADs act as an ecological trap for tunas. Fréon and Dagorn (2000) reviewed different hypothesis to explain the associative behaviour of some pelagic species and tunas in particular. For tunas the meeting point or the indicator-log hypotheses are the most feasible (Dagorn *et al.*, 2012). The meeting point hypothesis (Dagorn and Fréon, 1999) suggests that objects facilitate the encounter rate of specimens in order to constitute bigger schools which are more efficient for the survival of the species; the indicator-log hypothesis (Hall, 1992) posits that natural floating objects, which are originated from productive waters (e.g., rivers, mangrove swamps), drifted offshore and ocasionally become traped in frontal zones, are used by tunas to stay in contact with productive water masses.

4.4. Biology

4.4.1. Age and growth

Various methods have been used to evaluate the growth rate of skipjack tuna: analysis of cohort progression in length frequency distribution or modal progression analysis, analysis of the hard parts of the fish (e.g. otoliths, vertebra, and dorsal spines) also known as direct methods and analysis of tag and recapture data. It resulted in a wide variety of estimates of growth rate (Table 4.1).

Table 4.1. Skipjack growth rate (K) and maximum length (L∞) estimations in different areas and with different methods (i.e. Modal progression analysis, direct methods or Tag-release-and-recepture (*adapted from Murua et al.,In prep*)

Reference	Ocean	L∞	K	Method
Marcille and Stequert, 1976	Indian Ocean	60.6	0.93	Modal progression analysis
Hafiz, 1985	Central Indian Ocean (Maldives)	78.0	0.63	Modal progression analysis
Mohan and Kurnikoya, 1985*a*	Central Indian Ocean (Maldives)	90.0	0.49	Modal progression analysis
Hafiz, 1987	Central Indian Ocean (Maldives)	82.0	0.45	Modal progression analysis
Amarasiri and Joseph, 1987	Central Indian Ocean (Sri Lanka)	85.0	0.62	Modal progression analysis
Adam, 1999	Central Indian Ocean (Maldives)	64.3	0.55	Tag-release-and-recapture
Fonteneau and Gascuel, 2008	Indian Ocean	73.0	0.40	Tag-release-and-recapture
Hillary et al., 2008	Indian Ocean	76.0	0.29	Tag-release-and-recapture
Eveson et al., 2012	Indian Ocean	71.6	1.12; 0.33*	Tag-release-and-recapture
Koya et al., 2012	Northern Indian Ocean	92.0	0.50	Modal progression analysis
Brock, 1954	Central Pacific Ocean	85.0	0.95	Modal progression analysis
Joseph and Calkins, 1969	Eastern Pacific Ocean	72.9	0.83	Tag-release-and-recapture (not average)
Joseph and Calkins, 1969	Eastern Pacific Ocean	88.1	0.43	Tag-release-and-recapture (average)
Joseph and Calkins, 1969	Eastern Pacific Ocean	107.0	0.42	Modal progression analysis
Josse et al., 1979	Western Pacific Ocean	65.5	0.95	Tag-release-and-recapture
Josse et al., 1979	Eastern Pacific Ocean	79.1	0.64	Tag-release-and-recapture
Uchiyama and Struhsaker, 1981	Eastern and Central Pacific	102.2	0.55	Direct method (Otolith)
Wankowski, 1981	Central Western Pacific	74.8	0.51	Modal progression analysis
Sibert et al., 1983	Western Pacific Ocean	61.3	1.25	Tag-release-and-recapture
Brouard et al., 1984	Western Pacific (Vanuatu)	60.0	0.75	Modal progression analysis
Bayliff, 1988	Northeastern Pacific Ocean	96.3	0.52	Tag-release-and-recapture

* Two-stanza von Bertanlanffy growth model

Table 4.1. (suite)

Reference	Ocean	L∞	K	Method
Bayliff, 1988	South Eastern Pacific Ocean	66.5	1.82	Tag-release-and-recapture
Tanabe et al., 2003	Western Tropical Pacific (Japan)	93.6	0.43	Direct method (Otolith)
Wang et al., 2010	Western Central Pacific	70.7	0.64	Direct method (Spines)
Batts, 1972	North West Atlantic (North Carolina)	79.6	0.20	Direct method (Spines)
Bard and Antoine, 1986	Eastern Atlantic (Guinea)	80.0	0.32	Tag-release-and-recapture
Bard and Antoine, 1986	Eastern Atlantic (N. Trop)	80.0	0.60	Tag-release-and-recapture
Cayré et al., 1986	Northeastern Tropical Atlantic	62.0	2.08	Tag-release-and-recapture
Cayré et al., 1986	Northeastern Tropical Atlantic (Senegal)	57.7	2.96	Tag-release-and-recapture
Cayré et al., 1986	Northeastern Tropical Atlantic (Cap Vert)	59.9	1.54	Tag-release-and-recapture
Vilela and Costello,1991	Western Atlantic	87.1	0.22	Direct method (First fin-ray)
Pagavino and Gaertner, 1995	West Atlantic (southearten Caribean Sea)	94.9	0.34	Modal progression analysis
Hallier and Gaertner, 2006	Eastern Atlantic Ocean (Senegal)	97.3	0.25	Tag-release-and-recapture
Gaertner et al., 2008	Eastern Atlantic Ocean (S of 10ºN)	112.3	0.14	Tag-release-and-recapture
Gaertner et al., 2008	Eastern Atlantic Ocean (N of 10ºN)	89.4	0.38	Tag-release-and-recapture

However, growth still remains a key area of uncertainty (IOTC, 2011). Growth estimations by measuring hard parts and length frequency analysis did not result in reliable measures (Uchiyama and Struhsaker, 1981; Wild *et al*., 1995; Adam *et al*., 1996). On the one hand, Adam *et al.* (1996) observed that the micro increment deposition rate in otoliths was variable among specimens confirming that ring counts would not be suitable for daily growth increments estimation. Lack of food and reproductive events might be able to induce some breaks in growth that result in a reduction in the deposition of increments (Adam *et al.,* 1996). Recently this method was validated by the use of oxytetracycline in juveniles (Tanabe *et al*., 2003; Kayama *et al.*, 2007). On the other hand, the analysis of the length frequency data is of little value due to continuous recruitment, high and variable growth rate and migratory behaviour of the species (Joseph and Calkins, 1969; Josse *et al*., 1979; Wild and Hampton, 1994). Tagging could also be subjected to errors, such as the possible influence of the technique itself on fish growth and difficulties involved in recompilation of reliable recapture size (Sibert *et al.,* 1983; Yesaki and Waheed, 1992; Anderson *et al.,* 1996). In the same way, inaccuracy in the size at liberty can induce large shift in the estimation of growth parameters (Sibert *et al*., 1983). However, tagging was claimed to be the technique providing the best means of estimating growth rate in skipjack (Josse *et al*., 1979).

Since the 50s, tropical tuna tagging programs has been carried out worldwide by the RFMOs. In the Indian Ocean, the IOTC conducted pilot and small scale tagging activities as well as a large-scale Regional Tropical Tuna Tagging program (RTTP-IO) between 2002 and 2009 in which 101,212 skipjack were tagged (Hallier and Million, 2012). Since the 60s a total of 43,448 skipjack have been tagged in the Atlantic Ocean within the framework of different tagging programs conducted by the ICCAT. In the Eastern and Western Pacific, the number of tagged fish by SPC and IATTC rises to 131,227 and 620,148, respectively (Fonteneau and Hallier, 2012).

Tagging data provided valuable information of important parameters of the skipjack growth. It was observed that the skipjack growth rate at size is variable seasonally in a given region (Sibert *et al*., 1983) and spatially between latitudes (Sibert *et al*., 1983; Bard and Antoine, 1986; Bayliff, 1988; Gaertner *et al*., 2008), longitudes (i.e., Eastern and Western Pacific) (Sibert *et al*., 1983; Bayliff, 1988) and oceans (i.e., Eastern Pacific and Indian Ocean) (Fonteneau and Hallier, 2012), probably due to saptiotemporal variations in environmental conditions. For example, in the Atlantic Ocean growth parameters from the two groups of skipjack separated by 10ºN latitude could differ as much as 23 cm in maximum length (L_∞) and 0.24 in growth rate (K) per year (Gaertner *et al.,* 2008). Similar differences were observed between East and West Atlantic, where fishes in the Western Atlantic attain larger maximum length (Pagavino and Gaertner, 1995; Vilela and Castello, 1991; Matsura and Andrade, 2000). Most of the works on skipjack growth have been carried out in the Pacific Ocean before the 90s, the Indian Ocean being the least investigated (Murua *et al*., In prep). Moreover, a high variability of the growth parameters exists among works carried out in each basin (Table 4.1). Whether the differences are real or due to the method or sampling strategy applied is unknown. Studies made in the Pacific observed that linear models seem to be inappropriate for describing the growth of tuna species (Sibert *et al.,* 1983). In this sense, Uchiyama and Struhsaker (1981) found that growth function was best explained by a three stanza growth model based on otolith reading. Similarly, differences in growth rate were observed in the Indian Ocean between small and large specimens, with estimates of rapid growth for smaller tuna and fairly slower growth for medium and large ones (Maunder, 2002; Bruyn and Murua, 2008; Eveson *et al*., 2012). Eveson *et al.* (2012) observed a change in growth rate around 45 cm, equivalent to one year (Tanabe *et al*., 2003), from which the growth rate slows down. The growth could be diminished from a transfer of energy from somatic growth to gonad development (Maunder, 2002). In this sense, a two stanza-model could be more appropriate for skipjack (Eveson *et al.* 2012). Vilela (1990) observed that sexual dimorphism in growth was not significant.

4.4.2. Natural Mortality

The natural mortality is one of the essential parameters for stock assessment, but one of the most difficult to estimate (Adams, 1999; Wild and Hampton, 1994). Estimates of natural mortality can be obtained from different approaches: the use of catch and effort statistics, the measurement of the attrition of members of a specific year class as they pass through the fishery, and the quantification of the rate at which tagged specimens are recovered (Joseph and Calkins, 1969). Because skipjack stocks do migrate and catches are not possible to segregate into age groups, tagging experiments might bring the most reliable estimates (Joseph and Calkins, 1969). However, all methods are troublesome due to the emigration factor (Wild and Hampton, 1994). In addition, if tagging is used to estimate mortality rate, factors derived from the tagging itself (i.e. dies due to tagging effect and/or immediate and gradual tag loss) have to be considered. For skipjack, mortality due to tagging is unknown but might be significant and higher than in other tuna species (Wild and Hampton, 1994; Hoyle and Hampton, 2012).

Skipjack natural mortality rate may be high (Adam, 1999) and size dependent (Hampton, 2000). In the Pacific Ocean, natural mortality of small skipjack (FL < 40 cm) is one order of magnitude higher than medium-size tuna (i.e. size classes of 41-50, 51-60 and 61-70 cm with a natural mortality rate of 1.6, 1.2 and 2 year^{-1}, respectively). Nevertheless, large-size tuna (> 70 cm) also suffer from high mortality rate which might indicate the onset of the senescence (Hampton, 2000). Mortality rate estimates obtained by Adam and Sibert (2002) in Maldives were consistent with those obtained by Hampton (2000) for medium-size fish (i.e., 40-70 cm). Recently, Eveson (2011) estimated the skipjack mortality through the application of the Brown-Peterson method (developed by Polacheck *et al.*, 2006) to tag-recapture and catch data in the Indian Ocean. As observed previously, results suggest that natural mortality from 1 to 4 years was U-shaped, being the highest at ages 1 and 4 and lower at age 2 and 3. Significant variations in the mortality rate were observed between years (Eveson, 2011).

4.4.3. Reproduction

The skipjack is a gonochoristic fish (Matsumoto *et al.*, 1984); however some rare cases of hermafroditism have been described (Uchida, 1961; Pillai *et al.*, 2007). Mating is promiscuous and fertilization occurs externally (Matsumoto *et al.*, 1984). It does not present sexual dimorphism (Jones and Silas, 1963). Estimations of sex ratio are variable. A predominance of males was observed in the Indian Ocean during the reproductive active periods which was attributed to the need of surplus sperm production (Raju, 1964; Amarasiri and Joseph, 1987; Stéquert and Ramcharrun, 1996). A simiar pattern was observed in the Atlantic (Batalyants, 1989) and Pacific (Schaefer and Orange, 1956) Oceans. In addition, males seem to be more numerous at larger sizes than females due to a lower mortality rate and/or a particular behaviour making them less vulnerable to fishing (Raju, 1964; Stéquert and Ramcharrun, 1996; Timohina and

Romanov, 1986; Schaefer, 2001*b*). Schaefer (2001*b*) suggested that the high energetic cost associate to reproduction can have important consequences in female mortality. Some studies, however, observed either a predominance of females (Batts, 1972; Matsumoto *et al.*, 1984) or an equal number of each sex (Cayré, 1981; Cayré and Farrugio, 1986; Goldberg and Au, 1986) in the Atlantic Ocean.

The ova develop uniformly throughout the ovary and in both lobes (Stéquert and Ramcharrun, 1995). In females in spawning capable phase, oocytes of all stages are present with no dominant population. Therefore, the oocyte development is defined as asynchronous and, consequently, spawning occurs in multiple batches (Cayré and Farrugio, 1986; Goldberg and Au, 1986; Stéquert and Ramcharrun, 1996, Schaefer (2001*a*, 2001*b*, 2001*c*). Testes are considered as unrestricted spematogonial testis type because the distribution of spermatogonia occurs along the entire tubule (Schaefer, 2001*b,* 2001*c*). A detailed histological description of skipjack testes is provided by Ashida *et al*. (2010). Little is known on the reproductive strategy adopted by the species (i.e. indeterminate or determinate fecundity regulation) (Murua and Saborido-Rey, 2003). Previous studies hypothetised an indeterminate fecundity (Cayré and Farrugio, 1996; Stéquert and Ramcharrun, 1995; Stéquert and Ramcharrun, 1996; Timohina and Romanov, 1996), but without analyzing the lines of evidence defined by Hunter *et al.* (1992), Greer Walker *et al.* (1994), Murua and Saborido-Rey (2003) to validate the oocyte growth and recruitment pattern.

Although the length at 50% maturity has been largely investigated for skipjack (Table 4.2), diverse methods were used and no standard classification criteria have been established resulting in variable predictions. Using histological classification, the length at 50% maturity was 41-42 cm and 42-43 cm in females and males from the Western Indian Ocean, respectively, corresponding to fish of 1.5 years (Stéquert and Ramcharrun, 1996; Timohina and Au, 1996).

Table 4.2. Worldwide estimations of the length (L_T: total length; L_F: fork length) at 50% of maturity for skipjack tuna females (F) and Males (M)

Reference	Area	F	M	Method	Type of length measure
Raju, 1964	Central Indian Ocean (Minicoy islands)	40-45*		Oocyte size distribution, gonadosomatic index	L_T
Stéquert, 1976	Western Indian Ocean (Nordwest Madagascar)	41-43		Histology	L_F
James et al., 1992	Central Indian Ocean (Lackshadweep)	44-45		Macroscopic	L_T
Hafiz, 1986	Central Indian Ocean (Maldives)	35*		Macroscopic	L_F
Timohina and Romanov, 1986	Western Indian Ocean	43	40	Histology	--
Amarasiri and Joseph, 1987	Central Indian Ocean (Sri Lanka)	43	42	Macroscopic	L_F
Stéquert and Ramcharrun, 1996	Western Indian Ocean	41-42	42-43	Histology	L_F
Norungee and Kawol, 2011	Western Indian Ocean	43	44	Macroscopic	L_F
Koya et al., 2012	Central Indian Ocean (Lackshadweep)	41		Macroscopic	L_F
Batts, 1972	Western Atlantic Ocean (North Carolina)	50		Histology	--
Cayré, 1981	Eastern Atlantic	44	46	Oocyte size distribution	L_F
Cayré and Farrugio, 1986	Atlantic Ocean	42	45	Histology	L_F
Goldberg and Au, 1986	Western Atlantic Ocean (Brazilian coast)	51*		Macroscopic	L_F
Vilela and Costello, 1993	Western Atlantic Ocean (Brazilian coast)	52	51	Macroscopic	L_F
Wade, 1950	Westen Pacific (Philippines)	39*	38*	Macroscopic	L_F
Brock, 1954	Pacific Ocean (Hawaii)	40-45*		Oocyte size distribution	--
Schaefer and Orange, 1956	Pacific	50*		Macroscopic	L_T

* Minimum size of skipjack at maturity

When sexually active, spawning occurs in offshore waters whenever the hydrologic conditions are favourable (Schaefer, 2001*b*). The latitudinal range of the spawning is delineated on the north and south by the 24ºC isotherm (Matsumoto *et al*., 1984; Cayré and Farrugio, 1986; Schaefer, 2001*a*, 2001*b*). Spawning seems to be independent from salinity and could be related to wind stress (Stéquert and Ramcharrun, 1996). In addition, fish does not appear to migrate towards discrete spawning areas (Hunter *et al*., 1986; Schaefer, 2001*a,* 2001*b*), although the possible existence of discrete breeding grounds was also reported in the Indian Ocean (Dammannagoda *et al.*, 2011; Menezes *et al*., 2012)**.** Spawning occurs throughout the year in tropical waters and seasonally in subtropical waters (Matsumoto *et al*., 1984). Spawning period becomes shorter as distance from the equator increases (Matsumoto *et al*., 1984). In the equatorial Western Indian Ocean whatever the month 70% of females were found in spawning capable phase between 10ºS and 10ºN (Stéquert and Ramcharrum, 1996). However, periods of higher sexual activity were defined: from November to March and from June to July, which correspond to North-east monsoon and South-west monsoon season respectively (Stéquert and Ramcharrun 1996; Timohina and Romanov, 1996; Stéquert *et al.,* 2001). A similar spawning pattern was observed in Indian waters by Koya *et al*. (2012) who identified year around spawning with a peak from December to March and a minor one during June to August. James and Pillai (1979) observed that in the Arabian Sea and Bay of Bengal mature specimen dominated the catches in January-February, whereas in the Central Indian Ocean the spawning activity was high from September to April. In Minicoy waters, extend spawning seasons from January to April and from June to September were described (Raju, 1964). In the same region, Mohan and Kuhnikoya (1985*b*) observed signs of spawning throughout the year. Amarasiri and Joseph (1986) found ripe ovaries in all months (except in November and December) along the northwest to south coast of Sri Lanka, with the highest proportion of mature and spawning females being caught from March to May and from June to July. Therefore, extended spawning seasons are predominantly observed in the Indian Ocean. For equatorial Eastern Atlantic Ocean, year around spawning was observed with the highest activity from January to April and from October to November (Cayré and Farrugio, 1986). Seasonal spawning was observed in higher latitudes (Cayré and Farrugio, 1986). In the Western Atlantic, the spawning period was defined from November to March (Goldberg and Au, 1986). In the Indian and Eastern Atlantic Oceans, Timohina and Romanov (1996) and Alekseev and Alekseeva (1986) attributed the year around spawning to the presence of different groups in equatorial waters that spawn at opposite phases. In Central Pacific, near the Hawaian islands, spawning is long lasting from February to September (Brock, 1984). In Philippine waters, ripe skipjack were caught through the year with intense spawning observed in June (Wade, 1950). In the Eastern Pacific, reproductive active fish were found north of the equator throughout the year and south of the equator during the first three quarters of the year without any seasonal peaks (Schaefer, 2001*a*).

On the other hand, Cayré (1981) and Cayré and Farrugio (1986) mentioned that the spawning could be synchronized at school level. In this sense, fish with ripe eggs which are rare in catches may adopt a distinct behaviour (Jones and Silas, 1963; Matsumoto *et al.*, 1984; Mahon and Kunhikoya, 1985*b*). Spawning appears to occur at night (Hunter *et al.*, 1986). Courtship behaviour is common prior to each spawning event which include pairing of individuals, rapid horizontal and vertical swimming and coloration changes (Schaefer, 2001*b*)

Compared to other Thunnus species, the skipjack batch fecundity is high (Schaefer, 2001*b*). The Table 4.3 reviews batch fecundity estimates for this species revealing quite variable data among oceans. For example, the batch fecundity estimate for fish with L_F of 40-70 cm reached 1,000,000 oocytes in the Atlantic Ocean (Cayré and Farrugio, 1986), 2-fold lower compared to the Pacific and Indian Oceans (Raju, 1964; Yoshida, 1966).

Table 4.3. Worldwide estimations of the batch fecundity (F_B) of skipjack. *n*= number of fish

Reference	Area	*n*	Size range (cm)	F_B (10^3 oocytes)
Raju, 1964	Central Indian Ocean (Minicoy islands)	63	41.8 - 70.3	151.9 - 1,978
Stequert, 1976	Western Indian Ocean	64	44.1 - 56.5	87.0 - 824.0
Mohan and Kunhikoya, 1985*b*	Central Indian Ocean (Minicoy islands)	23	46.5 - 66.0	170.5 - 682.9
Timohina and Romanov, 1986	Western Indian Ocean	7	52-69	906.0 - 2,773.3
Amarasiri and Joseph, 1987	Central Indian Ocean (Sri Lanka)	--	44-68	211.4 - 2,952.2
Uktolseja and Purwasasmita, 1991	Eastern Indian Ocean	7	40.3-47.4	543.0 - 1,282.9
Stéquert and Ramcharrun, 1995	Western Indian Ocean	281	44 - 75	80.0 - 1,250.0
Simmons, 1969	Tropical Atlantic Ocean	13	46.5 - 80.9	262.0 - 1,331.0
Batts 1972	Western Atlantic Ocean (North Carolina)	31	49.8 - 70.4	141.0 - 1,200.0
Cayré, 1981	Eastern Atlantic Ocean	71	43-65	100.0 - 900.0
Cayré and Farrugio, 1986	Atlantic Ocean	231	41-70	100.0 - 1,000.0
Goldberg and Au, 1986	Western Atlantic Ocean (Brasil)	24	43-78	129.8 - 977.6
Yabe, 1954	Pacific Ocean	5	46.8 - 61	113.3 - 859.9
Yoshida, 1966	Central Pacific Ocean	4	43 - 75	100.0 - 2,000.0

As shown in Fig. 4.9, batch fecundity seems to increase with body size; however, negative relationship between relative batch fecundity and fish length was also reported (Cayré and Ferrugio, 1986). A high intra-species variability of batch fecundity was reported for specimens of similar size (Cayré, 1981; Cayré and Farrugio, 1986; Goldberg and Au, 1986; Schaefer, 2001*b*). A strong spatiotemporal variability was also higlighted. For example, Cayré and Farrugio (1986) observed significant differences among regions from the Eastern Atlantic Ocean, where the batch fecundity increased towards the equator. In the Western Indian Ocean, relative batch fecundity estimates ranged from 40 to 130 eggs/g of body weight and showed a seasonal decrease during inter monsoon events (i.e. May, September and October) (Stéquert and Ramcharrun, 1995). Joseph (1963) and Cayré and Farrugio (1986) attributed such high variability to the decreased number of batch eggs with successive spawnings throughout the year. In general, the batch fecundity estimation relies on a small sample size (*n;* Table 4.3) mainly due to the difficulty to collect skipjack females in advanced stage of oogenesis. Sampling is usually restricted to day-light hours corresponding to the fishing time, whereas ovulation (occurring in 8 h; Kaya *et a*l., 1982) and spawning might occur in the afternoon and during night hours (Hunter *et al.,* 1986; Schaefer, 2001*b*). Hunter *et al.* (1986) estimated a mean spawning interval of 1.18 days based on the incidence of females with postovulatory follicles both on wild and captive skipjack in the Pacific Ocean. Based on an oocyte size frequency distribution analysis, Stéquert and Ramcharrun (1995) predicted that the Indian Ocean skipjack undergoes 4 successive spawnings per year, each batch formation lasting 40 to 60 days. According to them, the previous data obtained by Hunter *et al.* (1986) might be due to a partial oocyte ovulation in the batch. However, this hyphothesis remains untested and even seems unlike due to the oocyte recruitment and ovulation pattern described in teleosts (Murua and Saborido-Rey, 2003; Brown-Peterson *et al.*, 2011; Lowerre-Barbieri *et al.*, 2011*a*). The spawned eggs are transparent, buoyant with a single oil droplet of 0.8-1.2 mm (Stéquert, 1976; Matsumoto *et al.*, 1984; Cayré and Farrugio, 1986).

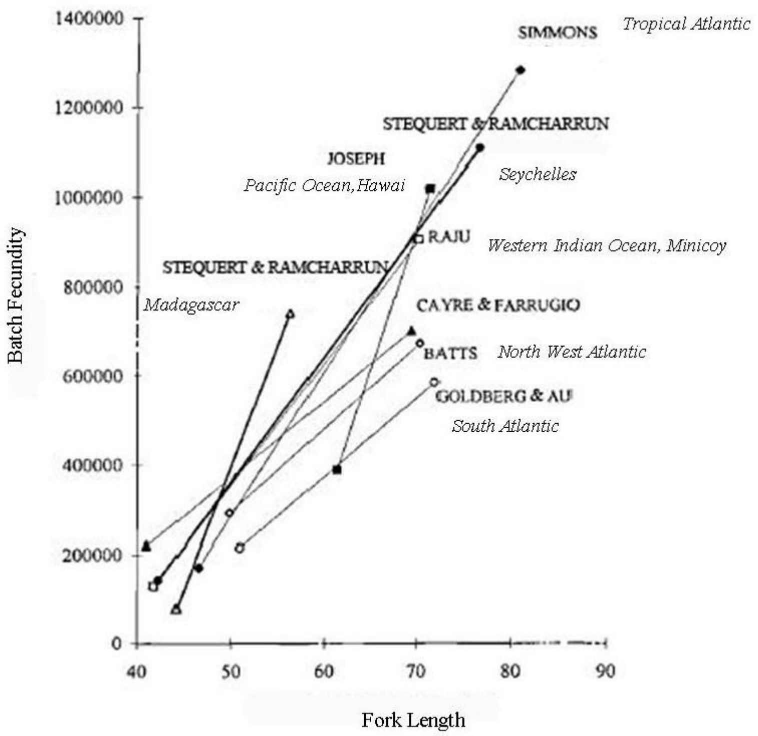

Fig. 4.9. Relationships between batch fecundity (number of oocytes) and fork length (cm) estimated by different authors for different areas in the Pacific, Atlantic and Indian Ocean (*adapted from Stéquert and Ramcharrun, 1995*)

4.4.4. Early life history and recruitment

After fertilization the development of the embryo is rapid and the larvae hatch in about 1 day (Matsumoto *et al.*, 1984). The incubation period is inversely related to the temperature. The hatched larvae total length is about 2.6 mm. In 2 days after hatching, the larvae absorbed their yolk sac and start feeding (Ueyanagi *et al.*, 1974; Matsumoto *et al.*, 1984).

The spatial and seasonal distribution of larvae is initially established by adult spawning and could be modified by passive processes (i.e. surface current) (Boehelrt and Mundy, 1994). Therefore, like spawning, the distribution is strongly influenced by water temperature (Matsumoto *et al.*, 1984; Juarez and Frias, 1986; Boehlert and Mundy, 1994). Larvae are generally found in waters with temperature over 24 to 25ºC (Conand and Richards, 1982; Matsumoto *et al.,* 1984), but, sampling have also been reported in South Atlantic waters cooler than 24ºC (Kikawa and Nishikawa, 1980).

Moreover, Forsbergh (1989) stated that larvae concentration doubled with a 1ºC-increase in the Pacific waters from 23 to 29ºC.

The larvae are distributed widely along the three oceans (Nishikawa *et al*., 1978). In the Atlantic and Pacific, responding to the westward warm equatorial current flow and cold eastward currents at high latitudes which deflects towards the equator, the distribution is broad latitudinally in the west but narrow in the east (Matsumoto *et al*., 1984). In the Western Pacific, larvae has been found as far as 35ºN and 37ºS, while in the eastern the distribution is constrained to 15ºN and 5ºS (Matsumoto *et al*., 1984). In the West Atlantic, the northern boundary was established at 30ºN and the southern at 25ºS (Kikawa and Nishikawa, 1980; Matsumoto *et al*., 1984), while the eastern boundary seems to be fairly narrower (Richards and Simmons, 1971). In contrast, larvae from the Indian Ocean were caught as far as 30ºS in the east, 36ºS in the west with most of the observations north of 20ºS (Ueyanagi, 1969; Conand and Richards, 1982), the northern boundary being limited by the Asian Continent. According to Conand and Richards (1982) and George (1990), the North Mozambique Channel from November to April as well as waters off the South-western Indian coast from February to April and June corresponds to areas of particular high larvae abundance.

Boehelrt and Mundy (1994) and Caveriviere *et al*. (1976) observed high abundance of skipjack larvae in offshore waters around Hawaian islands and from the eastern equatorial Atlantic respectively, while near shore occurrence was reported in waters off the South-western Indian coasts (George, 1990). Skipjack larvae are restricted to the mixed layer and they occupy deeper waters than *Thunnus spp*. and *Auxis spp.* (Boehelrt and Mundy, 1994). Boehelrt and Mundy (1994) did not observe skipjack larvae deeper than 84 m, with the highest density recorded at 40 m. Skipjack larvae may carry out vertical migration, but generally within the mixed layer (Klawe, 1963; Davis *et al*., 1990). Davis *et al*. (1990) observed movements towards deeper waters during daytime, and Kikawa and Nishikawa (1980) hypothesized that movements towards the surface could occur at night. However, while Boehlert and Mundy (1994) did not observe significant differences in the vertical distribution between day and night times, they noticed a negative relationship between larvae size and depth. On the other hand, George (1990) defined a favourable salinity range between 34 and 36, an opposite result from the ones of Conand and Richards (1982) and Caveriviere *et al*. (1976) for who either no relationship exists or in a wide salinity range (from 31 to 37). The scombrid larvae are in general voracious feeders (Hunter and Kimbell, 1980; Olson and Scholey, 1990).

The metamorphosis from larvae to juvenile occurs at approximately 12 mm (Tanabe *et al.,* 2001). The ecology of the skipjack during juvenile and young stages is largely unknown due to the low fishing efficiency of plankton nets. Tanabe and Niu (1998) and Tanabe *et al.* (2001) designed a new net (i.e. TANSYU) to gain insights into the early life story of skipjack. They succeeded in fishing juveniles ranging from 5.5 to 117.6 mm, but were unable to catch larger fish. Therefore, the most important information on these stages came from specimens collected from billfish and tuna stomach contents (e.g. Yoshida, 1971 and Mori, 1972). The growth rate of skipjack juveniles is high, approximately 2 cm per month (Yoshida, 1971; Tanabe *et al.,* 2001). It must be noticed that at length of 20 mm, the swimbladder of skipjack larvae degenerates (Richards and Dove, 1971; Matsumoto *et al*., 1984). The distribution of juveniles seems to be similar to that of larvae (Yoshida, 1971; Matsumoto *et al*., 1984; Wild and Hampton, 1994) which generally coincides with the spawning area (Tanabe *et al*., 2001). Tanabe *et al* (2001) observed a higher abundance of juveniles between depths of 40 and 120 m in the Western Pacific Ocean. As for larvae, skipjack juveniles reach deeper waters than does other tuna species (i.e. *Thunnus spp*) and supports lower temperatures (Tanabe *et al*., 2001). The vertical distribution range decreases as the juveniles develop and their swimming ability improves. In this sense, early juveniles (size < 10 cm) appear at wider depths, from the near surface to 200 m, than larger ones found at depth of about 50 m (Tanabe *et al.,* 2001). On the other hand, at late young stages (size between 15 and 35 cm), fish started to migrate between regions (Yoshida, 1971; Mori, 1972; Tanabe *et al*., 2001). In the Pacific Ocean, migrations towards higher latitudes have been observed (Yoshida, 1971; Mori, 1972).

The protracted spawning season of skipjack enables a nearly continuum recruitment throughout the year (Yoshida, 1971; Andrade and Santos, 2004). However, seasonal peaks in juvenile abundance and modes in the length frequency distribution were observed in Hawaii during summer and fall seasons (Yoshida, 1971). In the South-Western Atlantic, a seasonal peak of recruitment to fishery ground was also reported. At the beginning of the year, the contribution of the young skipjack to the fishery increased, and weaker peaks were observed year around (Andrade and Santos, 2004). In the Central Pacific juvenile abundance was high from October to March (Argue and Kearney, 1983). Langley and Hampton (2008) reported a strong influence of the climatic events in the Western Central Pacific with peaks of abundance following El Niño and lower recruitment following La Niña. In the Indian Ocean, the recruitment of juveniles to the fishery is relatively stable (IOTC, 2012), the low variations being due to environmental conditions (Langley and Hampton, 2008). However, the seasonal variability may be higher far from the equator compared to tropical waters where skipjack recruitment occurs continuously year around (Andrade and Santos, 2004).

4.4.5. Trophic ecology

Skipjack is considered as a day feeder (Roger, 1994*a*). Nakamura (1962) who studied fish in captivity observed that fish have difficulties in seizing food items during night time. In this sense, prey organisms available to skipjack tuna are those which remain in the 0 to 200 m layer during the day (Roger, 1994*a*). The diurnal fluctuations in feeding seem to be marked by food availability and satiation (Dragovic, 1970). Dragovic (1970) observed a higher percentage of empty stomachs near midday which was attributed to movements of forage to deeper layers due to maximum illumination or satiation of skipjack after the morning feeding. Although rare for this species, some evidence of night feeding in deep scattering layer (DSL) have been observed (Schaefer and Fuller, 2005; Romanov *et al*., 2009). On the other hand, field studies in tropical waters reported 85% of empty stomachs for skipjack tuna caught under Fish Aggregating Devices (FADs) anytime during the day (Ménard *et al*., 2000*;* Potier *et al*., 2001; Potier *et a*l., 2002; Jaquemet *et al*., 2011), hypothesing that skipjack might not feed under drifting FADs (Ménard *et al.,* 2000; Jaquemet *et al*., 2011).

Skipjack tuna are opportunistic feeders meaning they prey on any available forage (Matsumoto *et al*., 1984). Consequently, their diet is relatively diversified with more than 40 families of representative food items recorded in stomach contents (Alverson, 1963; Jaquemet *et al*., 2012). Skipjack preys are classified in three major categories, i.e. fishes, crustacea and molluscs, the contribution of each category to the total food items depending on spatiotemporal factors (Alverson, 1963; Dragovic, 1970; Dragovic and Potthoff, 1972; Ankenbrandt, 1985; Potier *et al*., 2002). For example, fish dominated

Atlantic skipjack diet with a dominance of Scombridae and of the mesopelagic fish *Vinciguerria nimbaria* in the Central and Eastern Atlantic waters respectively (Dragovic, 1970; Dragovic and Potthoff, 1972; Ménard *et al.*, 2000; Ménard and Marchal, 2003). In contrast, crustacea (i.e. Euphasidae and Galatheidae) were the main prey items recorded in Pacific skipjack stomach contents (Alverson, 1963).

In the Indian Ocean, long-term dietary changes have been observed shifting from fishes to crustacea (Potier *et al.*, 2008). Roger (1994*a*) observed a dominance of epipelagic fish, while a predominance of crustacea was reported in posterior works (Potier *et al.*, 2001; Jaquemet *et al.*, 2011). Epipelagic Engraulidae (i.e. *Engraulis japonicus*) and Nomeidae (i.e. *Cubiceps pauciradiatus)* are common fish prey in skipjack which constitute seasonally a important link in the transfer of energy between low and high trophic levels (Potier *et al.*, 2002; Potier *et al.*, 2008). On the other hand, Potier *et al.* (2001, 2002) and Jaquemet *et al.* (2011) recorded megalopoda larvae in relatively high abundance, and small size stomatopod (i.e. *Natosquilla investigatoris*) specifically from May to October. Cephalopods and other molluscs such as pteropods (*Cavolinia sp.*) appeared occasionally (Potier *et al.*, 2001; Jaquemet *et al.*, 2011). Finally, while canibalism on juvenile tunas has been reported in the Atlantic and Pacific (Dragovic, 1970; Dragovic and Potthoff, 1972; Lizuka *et al.*, 1989), it seems to be low in the Western Indian Ocean (Potier *et al.*, 2002).

Skipjack feed on small preys (Dragovic and Potthoff, 1972; Roger, 1994*a*; Jaquemet *et al.,* 2011). For example, fish preys from 32 to 42 mm were observed in skipjacks captured by purse-seine (Roger, 1994*a*). The influence of skipjack size on its prey size is not clear: Dragovic (1970) did not find any relationship whereas increased crustacea prey size was reported with increased predator size caught under FADs (Jaquemet *et al.*, 2011). Other studies showed an increase of the relative importance of fish prey with skipjack growth (Alverson, 1963; Dragovic and Potthoff, 1972; Ankenbrandt, 1985).

Since forage fish feed on macrozooplankton (i.e. copepods, amphipods, *Lucifer,* ostracods, magalopes and chaetognaths) (Dragovic, 1970; Roger 1994*b*), size ratio between tuna-prey and fish-plankton is very high suggesting that skipjack benefits from a short food chain (Roger, 1994*b*). Consequently, tuna schools probably concentrate where high densities of their prey feed on large zooplankton biomass concentrations, which at the same time aggregate where phytoplankton abundance is high (Roger, 1994*b*; Fonteneau *et al*., 2008). Good foraging conditions for tunas have been observed at cyclonic eddies, along the edge of eddies (Fonteneau *et al.,* 2008, Tew Kai and Marsac, 2010) and oceanic fronts (Mugo *et al.,* 2010).

Generally FADs are characterised by mixed schools of young yellowfin and skipjack that feed on similar prey items (Dragovic and Potthoff, 1972; Matsumoto *et al*., 1984; Jaquemet *et al*., 2011), with a competitive advantage for small yellowfin (Jaquemet *et al*., 2011). Other species such as kawakawa (*Euthynnus affinis*), dolphin fish (*Coryphaena hippurus*) and rainbow runner (*Elagatis bipinnulata*) are potential competitors (Matsumoto *et al*., 1984), and predators correspond to tuna and billfish species (e.g., yellowfin tuna, blue and white marlin and spearfish; Kikawa and Nishakawa, 1986).

4.4.6. Life history strategy and energy allocation

The life history strategy adopted by the species determines the energy balance between growth and reproduction, in particular how the animal should allocate resources to maximize the lifetime production of offspring (Wootton, 1984; Stearns, 1992). Life history strategies adopted by tunas are diverse (Juan-Jordá *et al.,* 2012), and skipjack lies towards the strategy described as “opportunistic” (early maturation, rapid growth and high energy investment in reproduction (Schaefer, 2001*b*; Fromentin and Fonteneau, 2001; Essington, 2003; Juan-Jordá *et al*., 2012). Essington (2003) predicted that skipjack allocates as much as 20-24% of the consumed energy to production (i.e. somatic and gonad growth) which refers to the storage fraction in an energy-balance equation (Wootton, 1998). Focusing on reproduction itself, calculations made by Hunter *et al.* (1986) determined average spawning daily cost of 1.7%. This energy invested to reproduction can be directly derived from the current feeding (i.e. income breeder) or indirectly and be financed by the energy stores (i.e. capital breeder) (Stearns, 1989). Energy allocation rules are usually governed by environmental conditions, physiological requirements and the reproductive strategy adopted by the species (Stearns, 1989; Saborido-Rey *et al*., 2010). In general, fish with high growth rate before maturation, early maturation, short or medium longevity, asynchronous oocyte development and indeterminate fecundity inhabiting temperate or tropical environments rely on current energy income for reproduction (Saborido-Rey *et al*., 2010). However, the skipjack energy allocation strategy remains unexplored.

Variation of the tissue proximate composition or energy content during reproductive cycle reflects the energy allocation strategy adopted by the species. Lipid and their constituent fatty acids constitute the major energy source for reproduction and larval development in fish, followed by proteins and carbohydrates (Sargent, 1995; Wiegand, 1996; Tocher, 2003). The published works on skipjack directed to measure the chemical composition of reproductive and somatic tissue are mainly focused on the quantification of polyunsaturated fatty acid content (PUFAs), i.e. omega 3 fatty acids known for its effects in protecting against human heart disease (docosahexanoic acid, DHA, and Eicosapentaenoic acid, EPA) (Medina *et al.,* 1995; Hiratsuka *et al.,* 2004; Intarasirisawat *et al.,* 2011). Indeed, tunas are characterised by high amounts of these PUFAs due to a selective catabolism which contributed to the accumulation of DHA and EPA (Saito et al., 1997; Tocher, 2003). However, the influence of gonad development on the biochemical composition of somatic and reproductive tissues has not been investigated for skipjack tuna.

5. Study area

The Indian Ocean is the smallest of all oceans. It has a north-south extent of 9,600 km from the Antartica to the Bay of Bengal and 7,800 km in the east-west direction between the east coast of Africa and west coast of Australia (Tomczak *et al.*, 2003). The main depth is of 3,800 m (Tomczak *et al.,* 2003). The continental selves are narrow, averaging 200 km. The only large self area is the Northwest Australian shelf, where shelf width exceeds 1,000km (Tomczak et al., 2003). Annual mean precipitations are low in the Western Indian Ocean north of 10ºS (i.e. 10 cm per year) and high in the East (i.e. 300 cm per year). However in the Southern Indian Ocean the inverse situation occurs: little rain in the east and high precipitations in the west. The variations in Sea Surface Salinity (SSS) follow the precipitation-evaporation balance which is marked by monsoon regimes (see next section). SSS in the eastern tropical region is fairly constant near and below 34.5. SSS increase toward the west and north into the Arabian Sea where annual mean reaches 36.0. The Sea surface temperature (SST) is high in the entire Northern Indian Ocean with mean temperatures above 28ºC. The SST seasonal variations are also related with monsoons (see next section). This study is mainly based on the Western side of the Indian Ocean and, thus, below the circulation and oceanographic conditions of the Indian Ocean, with special emphasis in the Western side, is described.

Western Indian Ocean circulation and Oceanographic conditions

South of 10ºS the ocean circulation does not reveal much seasonal variability and oligotrophic areas are manifested to the south (Wieggert *et al.*, 2006). The southeast trades persist throughout the year (Schott and McCreary, 2001). Driven by trades, the South Equatorial Current (SEC) inflow supplies the western boundary currents east of Madagascar within the latitude range 12–25°S (Fig. 5.1). At about 17°S, the SEC splits into northward- and southward-flowing branches. The southern branch turns southward and merges into the South Equatorial Madagascar Current (SEMC). The water mass flows into the retroflection zone at the southern end of Madagascar, from where there is a partial continuation towards the Aghulhas Current and partial return within the subtropical gyre. The northern branch, known as the Northeast Madagascar Current (NEMC), flows past the northern tip of Madagascar at Cape Amber and feeds into the

East African Coast Current (EACC). Subduction occurs mainly in the Southern Hemisphere.

The seasonally reversing monsoon wind system dominates the ocean climate north of about 10 ºS (Schott and McCreary, 2001). During the northern hemisphere summer (June-September) the South-west monsoon (SM) determines the ocean circulation (Fig. 5.1) and climate. Southwest winds blow steadily along east African coast. In this situation, SEC and EACC supply the northward flowing Somalia Current (SC). In the northern hemisphere SC turns offshore into the Southern Gyre (SG) and Great Whirl (GW) gyre. Associated to SG and GW, upwellings occur at about 3º-4ºN and 5-12ºN. Off the Arabian Peninsula and east and west of the Indian coasts weaker upwellings take place. The upwelled waters are carried far offshore (more than 500 km) and propagate southward by the Ekman circulation which is southward. When the Ekman flow approaches the equator, the winds and the meridional currents on the equator are northward. Under this circumstance, in the 5-10ºS Ekman divergence region open-ocean upwellings on the upwind and downwelling on the downwind side occur (Schott *et al.*, 2002; Hermes and Reason, 2008). As a result of the upwellings and high wind velocities, nutrient are advected over a large area of the Northwest Indian Ocean generating phytoplankton blooms (Veldhuis *et al.*, 1997; Wieggert *et al.*, 2006). Phytoplankton blooms occur from the coast of Somalia to Arabian Peninsula, Central Arabian Sea, Bay of Bengal and at the equator westward of 57ºE and between 5-15ºS (Veldhuis *et al.*, 1997; Wieggert *et al.,* 2006). Advection of upwelled waters reduces SST in the Western region of the Indian Ocean to 25-27ºC, with temperatures below 20ºC at the SC regions (Tomczak *et al.*, 2003). As with the winds and currents, SM produces the strongest rainfall (600 cm per year) in the Western Indian coast and Arabian Peninsula. As response, SSS decrease in the northern subtropics where they reach values of 33 (Tomczak *et al.,* 2003).

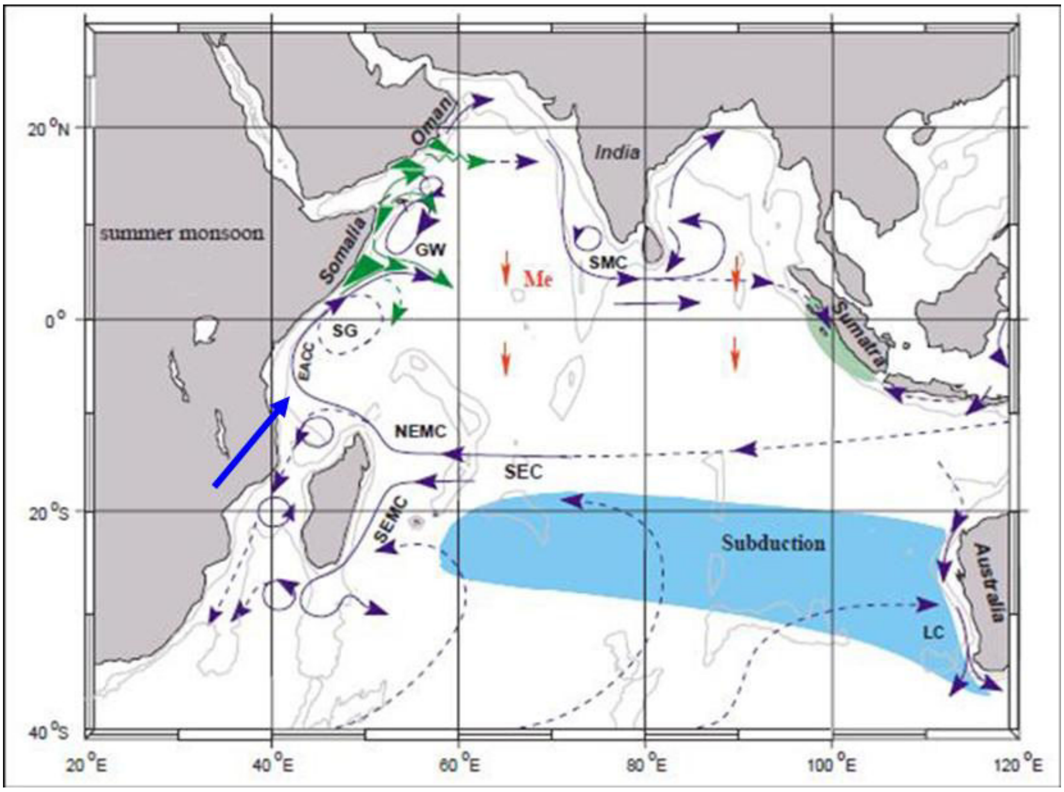

Fig. 5.1. Horizontal and shallow meridional circulation of the Indian Ocean during the South-west monsoon (SM). Currents branches: Southeast and Northeast Equatorial Mozambique Current (SEMC and NEMC), South Equatorial Current (SEC), South Equatorial Countercurrent (SECC), East African Coast Current (EACC), Somalia Current (SC), Souther Gyre (SG) and Great Whril Gyre (GW), Southwest Monsoon Current (SMC), and Leeuwin Current (LC). Southward Ekman transport indicated with red arrows, upwelling areas with green and subduction zone with blue and the wind direction with a blue arrow (*adapted from Schott et al., 2002*).

During the northern Hemisphere winter (December-March) the situation is reversed (Fig. 5.2). The winds are directed away from the Asian continent. Northeasterly winds make the Somalia current flows southward, which meet the EACC in a confluence zone at 2-4ºS. Both supply water of the east ward-flowing South Equatorial cuntercurrent (SECC). Beneath the surface, northward flow across the equator continues in a Somalia Undercurrent. During the season, the cold monsoon winds cool the waters. Augmented in salinity subduction take place in the Arabian Sea which will contribute supplying the upwelling zones (Fig.5.2) (Schott and McCreary, 2001; Schott *et al*., 2002). At the equator the circulation is reversed if compared with the SM. The Ekman transport is now northward at both sides of the equator and the meridional winds are southward generating open-ocean upwellings in the divergence zone. Phytoplankton blooms have been registered in the north and central Arabian Sea, Northern and Western Bay of Bengal, in the 5-10ºS Ekman divergence region. However, in general WM blooms are weaker than the ones observed in the SM (Wieggert *et al*., 2006).

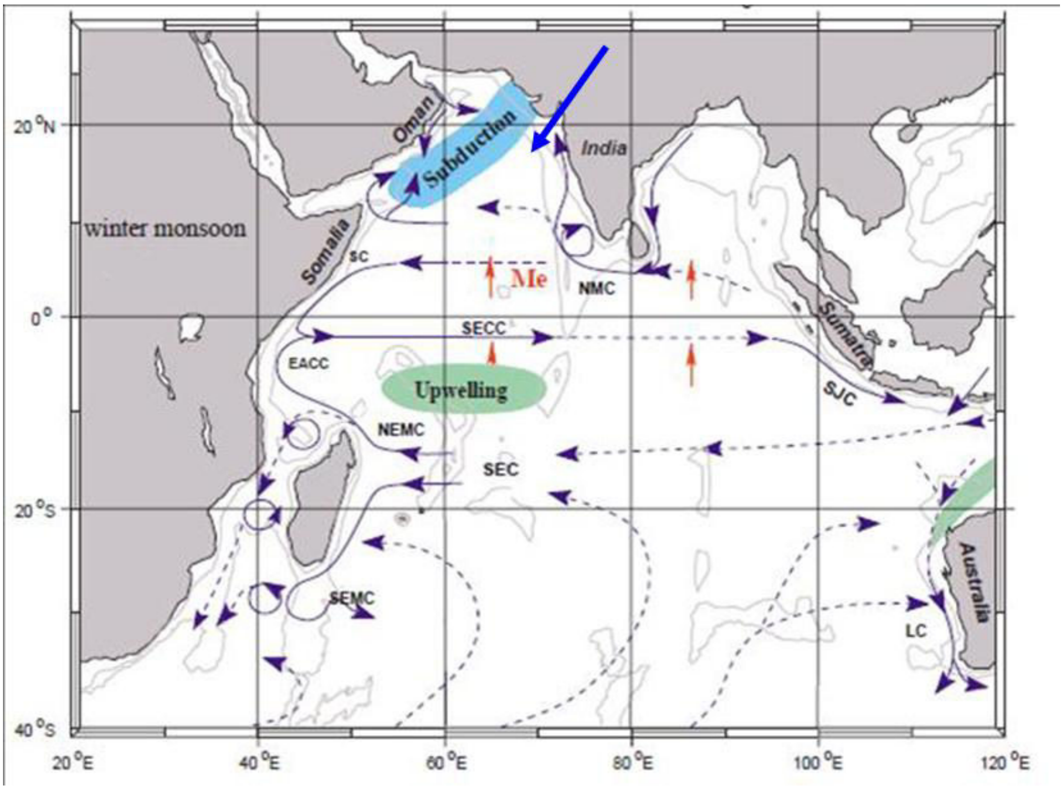

Fig. 5.2. Horizontal and shallow meridional circulation of the Indian Ocean during the North-East monsoon (NM). Currents branches: South Equatorial Mozambique Current (SEMC), South Equatorial Current (SEC), South Equatorial Countercurrent (SECC), East African Coast Current (EACC), Somalia Current (SC), Northeast Monsoon Current (NEMC), South Java Current (SJC) and Leeuwin Current (LC). Northward Ekman Transport in both sides of the equator is indicated with red arrows and the wind direction with a blue arrow (*adapted from Schott et al., 2002*)

During the transition seasons between the monsoons, i.e. April to June and October to December, a unique wind forcing occurs over the Indian Ocean. Semi-annual eastward winds over the equator generate an eastward annual-mean equatorial wind stress. In this situation, unlike the equatorial upwellings observed in other tropical oceans, the Indian Ocean is characterized by the highest SST (above 28ºC everywhere north of 10ºS) and a convergence zone along the equator increasing the thickness of the mixed-layer in the east (Schott and McCreary, 2001; Tomczak *et al*., 2003). In this situation biological activity generally is low (Murtugudde *et al*., 1999; Wieggert *et al*., 2006).

While Ekman transport is usually unidirectional in both sides of the equator during monsoon events, occasionally equatorial divergence can occur (Schott *et al*., 2002). In this situation the Indian Ocean resembles other large Oceans. Between 1993/94 and 1997/98 westward winds along the equator exerted upwellings in the east of the Indian

Ocean basin and downwelling in the west (Murtugudde *et al*., 2000). Cooler than normal SST off Sumatra and droughts occur in Indonesia, while warmer than usual SST were manifested over the western basin and severe rainfall in the eastern Africa. This situation can be triggered by the El Niño Southern Oscillation (ENSO), but can occur independently (Murtugudde *et al.,* 2000; Schott *et al*., 2009). Inter-annual ocean atmosphere interaction cause this climate variability identified as the Indian Ocean Dipole (IOD) (Saji *et al*., 1999).

Finally, the Mozambique Channel works as a southward water mass route. When NEMC encounters EACC in the northern tip of the Mozambique Channel, not all the water flow crosses the equator with the Somalia Current. While the majority of the surface flow follows EACC and SC, the subsurface flow pass southward through the Channel (Fig. 5.3) (Di Marco *et al*., 2001; Schott *et al*., 2002). The flow through the Channel may occur by mean of western boundary current along the continental slope (i.e. Mozambique Current MC) and anti-cyclonic eddies (MCE) passing through the channel (De Ruijter *et al*., 2002; Schouten *et al*., 2003). Annually on average, 4 anti-cyclonic eddies ranging from 300 to 350 km in diameter are observed to propagate southward through the Mozambique Channel (Schouten *et al*., 2003). The formation of eddies may result from southward advection of anomalies generated by Rossby waves coming from the east. This process is irregular and it is unlikely that it responds to a seasonal signal (Schouten *et al*., 2003; Ridderinkhof and De Ruijter, 2003). However, Riddenrinkhof *et al*. (2010) observed a correlation between the inter-annual variability and periods of IOD. The flow and SEMC are both sources for the Agulhas current and control the timing and frequency of Agulhas ring shedding into the Atlantic (Schouten *et al*., 2003).

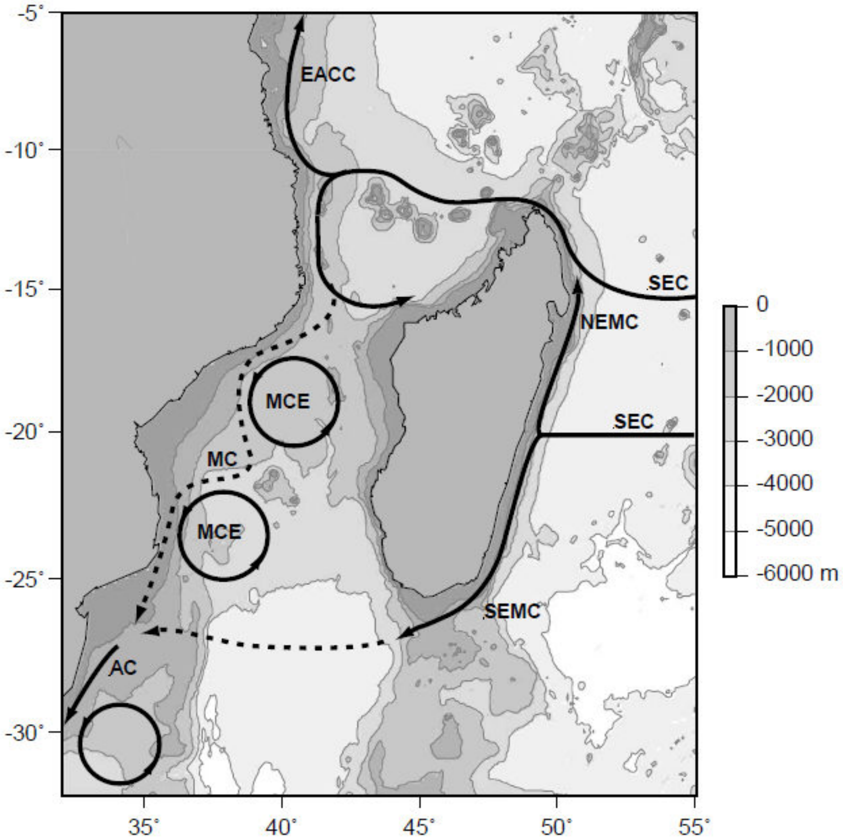

Fig. 5.3. Bathymetry and main currents of the Mozambique Channel: South Equatorial current (SEC), Northeast and Southeast Madagascar Currents (NEMS and SEMC), East African Coastal Current (EACC), Agulhas Current (AC), Mozambique Channel Eddies (MCE) and the Mozambique Current (MC) (*from Schouten et al., 2003*)

Anti-cyclonic eddies generate and upward movement of nutrient rich waters around their edge and advect nutrient rich coastal water when they run along the coast (Quartly and Srokosz, 2004; Tew Kai and Marsac, 2008). In this sense, eddy-induced upwellings or eddy-eddy interaction drive the distribution and transport of nutrients and support the food chain in the Mozambique Channel (Quartly and Srokosz, 2004; Sabarros *et al.,* 2009; Tew Kai and Marsac, 2010).

6. Chapter I

Oocyte development and fecundity type of the skipjack, Katsuwonus pelamis, in the Western Indian Ocean

Introduction

The skipjack (*Katsuwonus pelamis*) is the primary target species of the tuna and tuna-like species fishery, and represents around 58% of the total tuna catch worldwide (4.2 million during the last few years) (Arrizabalaga et al., 2012; ISSF, 2012). In the Indian Ocean, the catch of tropical tuna was around 0.85 million tons in 2008 (Restrepo, 2010) of which around 0.5 million tons was skipjack, mainly taken in the purse-seine fishery (IOTC, 2010). These figures show the importance of skipjack to the fishing sector in the Indian Ocean. Although no quantitative assessment is currently available in the Indian Ocean, the species is resilient and not prone to overfishing, due to its high productivity (IOTC, 2010).

In the Atlantic and Pacific Ocean several authors have studied the reproduction of this species: Schaefer and Orange (1956), Batts (1972), Matsumoto *et al.* (1984), Cayré and Farrugio (1986), Batalyants, (1989), Schaefer (2001*a*). In the case of the Indian Ocean, although there are few documents dealing with skipjack reproduction, the available information has, to a large extent, not been updated (Raju, 1964; Stéquert, 1976; Goldberg and Au, 1986; Stéquert and Ramcharrun, 1995; Stéquert and Ramcharrun, 1996; Timohina and Romanov, 1996; Stéquert *et al.*, 2001). However, in order to provide good scientific advice for the management of this species in the Indian Ocean, the reproductive biology of the skipjack in the Western Indian Ocean needs to be accurately investigated.

The skipjack, like most fish species of commercial interest, is an iteroparous and gonochoristic fish. It exhibits external fertilization without parental care. Previous studies have classified the skipjack as a multiple batch spawner fish with asynchronous oocyte development in which oocytes of all stages are present in the ovary with no dominant population present (Cayré and Farrugio, 1986; Goldberg and Au, 1986; Stéquert and Ramcharrun, 1996; Schaefer, 2001*a*, 2001*b*, 2001*c*). The spawning season is extended, which is commonly seen in warm water habitats. Spawning takes place all year around with periods of more intensive sexual activity during the North-east monsoon (i.e. November to March) and South-west monsoon season (i.e. June to July) (Stéquert and Ramcharrun 1996; Timohina and Romanov, 1996; Stéquert *et al.*, 2001).

The two spawning peaks have been associated with two populations with opposite phases of sexual activity (Timohina and Romanov, 1996). However, the Indian Ocean Tuna Commission, which is responsible for the management of this resource, accepts the one stock hypothesis for the Indian Ocean (IOTC, 2010). Regarding the size at first maturity, the latest studies in the Indian Ocean have estimated the size at first maturity as within a range of 41 to 43 cm in females and 40 to 43 cm in males, corresponding with 1.5 years (Stéquert and Ramcharrun, 1996; Timohina and Romanov, 1996).

Little is known, however, of the reproductive strategy of this species in relation to the model of fecundity regulation (i.e. indeterminate or determinate fecundity regulation) (Murua *et al.*, 2003; Kjesbu, 2009). Prior studies, using histological data and based on the evidence of a continuous oocyte diameter distribution in the ova (Cayré and Farrugio, 1996; Stéquert and Ramcharrun, 1995; Stéquert and Ramcharrun, 1996; Timohina and Romanov, 1996), suggested indirectly that the skipjack shows indeterminate fecundity, which is common in warm water habitats. Nevertheless, even if this assumption is valid it does not necessarily indicate an indeterminate fecundity type (Murua and Saborido-Rey, 2003), as found in other fish species such as mackerel, *Scomber scombrus,* (Greer Walker *et al*., 1994) and pout, *Trisopterus luscus* (Alonso-Fernandez *et al.*, 2008).

Therefore, this assumption needs to be clarified before any fecundity estimation is made. In species with determinate fecundity, total fecundity is fixed before the onset of the spawning, and the annual realized fecundity is estimated when correcting the total fecundity with the atretic losses. In indeterminate species the batch fecundity, spawning frequency and duration of the spawning are indispensable parameters (Hunter *et al.,* 1985; Murua and Saborido-Rey, 2003; Kjesbu, 2009). However, in the case of the skipjack this estimation becomes more complicated due to the extended spawning season (all year around) and the difference between the fishing and spawning time (Hunter *et al.,* 1986), which makes fecundity estimates difficult due to the lack of fish at spawning or showing signs of spawning in the fishing sets. In this context, the egg production ichthyoplankton surveys, independent of the fishing activity, would help in the estimation of the spawning potential of this species which would enable management measures to be implemented (Bernal *et al.,* 2012). The selection of any egg production method depends on having knowledge of the reproductive strategy in relation to the fecundity type (Armstrong and Witthames, 2012). For example, the Daily Egg Production Method (DEPM) is commonly applied to indeterminate fecundity style species, although it can also be applied to determinate species, whereas the Annual Egg Production Method (AEPM) and the Daily Fecundity Reduction Method (DFRM) are applied to determinate species (Armstrong and Witthames, 2012).

The main objective of this study is to define, for the first time, the fecundity type of the skipjack of the Western Indian Ocean basin in terms of oocyte growth and recruitment pattern in order to test the hypothesis that the skipjack shows indeterminate fecundity. For this purpose, and in order to analyze the dynamics of the oocytes in the secondary growth stages (Brown-Petersen *et al.*, 2011; Lowerre-Barbieri *et al.,* 2011*a*), the histology and the recently implemented advanced image analysis methodology (Thorsen and Kjesbu, 2001) are used. The study is based on four main criteria provided by Hunter et al. (1992); Greer Walker *et al.* (1994) and Murua and Saborido-Rey (2003): (a) stage-specific and seasonal variation of oocyte size-frequency distribution; (b) seasonal variation in the percentage of different oocyte stages during the spawning season in individuals in the spawning capable phase; (c) seasonal variation in the mean diameter of the oocytes in the tertiary vitellogenesis stage in individuals in the spawning capable phase; and (d) incidence of atresia through the spawning season.

Materials and Methods

Fish were collected onboard a purse-seiner during three cruises in the Western Indian Ocean, covering the area of East South Seychelles, North-west Seychelles, Chagos, Somalia and the Mozambique Channel, between January 2009 and May 2010 (Fig. 6.1). The fish were caught from 6:00 to 18:00 hours. Sex was determined at sea, whenever possible, by examination of the gonads. Ovaries were weighed on board and a sub-sample of the posterior right lobule was collected and preserved in a solution of 4 % buffered formaldehyde for histological analysis and assessment of oocyte size-frequency distribution.

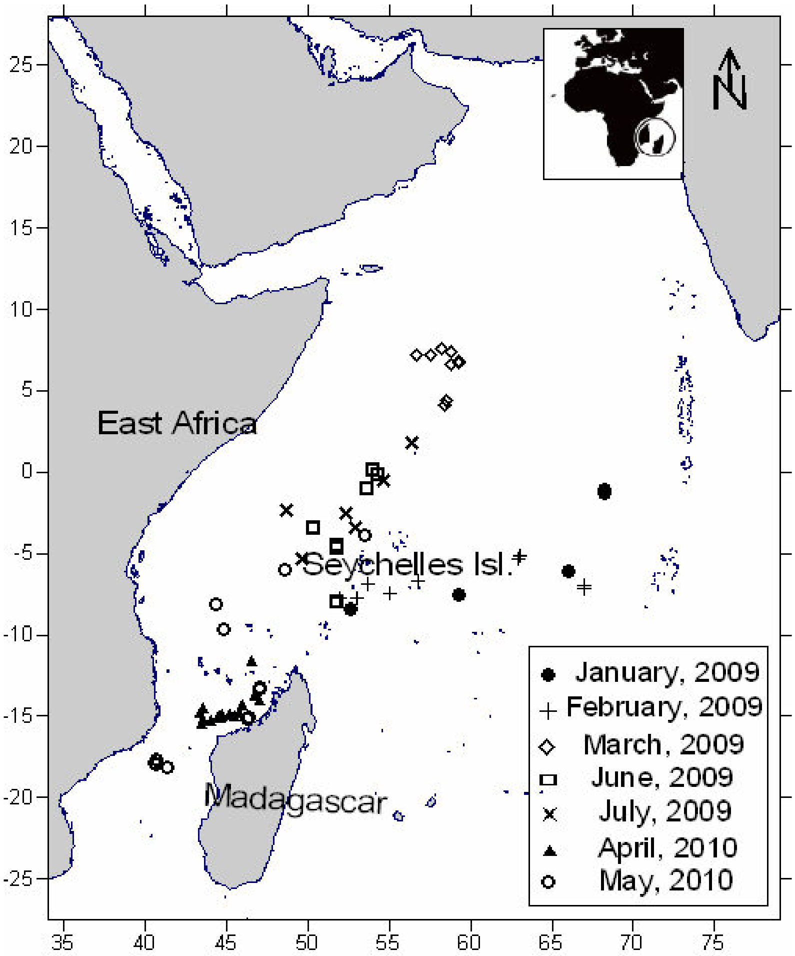

Fig 6.1. Sampling locations in the study area.

For histological analysis, cross-sections of about 5 mm of 673 female ovaries, fish ranging from 32 to 68 cm, were dehydrated by moving them through increasing concentrations of alcohol (i.e. from 70% to 96%) and soak in ascending solutions of resin. Then, the samples were polymerised into resin blocks. Histological sections of 3 to 5 µm were cut using a microtome and were stained with Harri´s Hematoxylin and Eosin staining.

Using the histological slides the ovaries were classified microscopically in different reproductive phases (Brown-Peterson *et al.,* 2011) according to the most advanced oocyte stage present in the gonad (West, 1990). Oocyte development stages were defined following the classification proposed by West (1990), Murua and Saborido-Rey (2003), and Lowerre-Barbieri *et al.* (2011*a*). From the histological sections, the incidence of atresia was also assessed (Hunter and Macewicz, 1985*a*). The oocyte resorption seems to follow a similar development process in all the teleosts (Hunter *et al.*, 1985) and, therefore, the atretic oocytes were staged based on the classification proposed by Hunter and Macewicz (1985*b*) for the northern anchovy. This has been proved to be the most useful classification and was used for the skipjack in previous works (Hunter *et al*., 1986). In the study only α and β stages were identified. The prevalence of atresia (i.e. the percentage of females showing some sign of α-atresia), together with the relative intensity of atresia (i.e. the percentage of α-atretic yolked oocytes in relation to total number of yolked oocytes) was estimated. Subsequently, the atretic condition of the ovary of each individual was established following the criteria defined by Hunter and Macewizc (1985*a*). In this sense, females in the regressing phase, i.e. females in the end of the reproductive cycle (Brown-Peterson *et al.,* 2011; Lowerre-Barbieri *et al*., 2011*a*), were identified when ≥50% of yolked oocytes were atretic (atretic states 2 and 3, i.e. incidence of atresia appears in ≥50% and 100% of the yolked oocytes, respectively). In contrast, actively spawning capable females (Brown-Peterson *et al.*, 2011), defined as those capable of spawning at the time of sampling or in the near future, were categorized as atretic states 0 (i.e. no yolked oocyte in atretic stage) and 1 (i.e. incidence of atresia in <50% of the yolked oocytes).

The samples preserved in formaldehyde were also used to analyse the frequency distribution of the oocyte diameters in 93 mature individuals (i.e. fish that would certainly spawn or had spawned showing the cortical alveoli stage or vitellogenic stage as the most advanced oocyte type present in the ovary, post ovulatory follicles or signs of atresia). These fish were chosen after applying the histological classification, in an attempt to cover all the ovarian developmental phases during the sampling period. Subsequently, whole-mount samples of these ovaries were weighed (i.e. 0.04 g to the nearest 0.0001g), and after being sieved they were covered with Rose Bengal stain (Witthames *et al*., 2009). After one day of exposure, the oocytes were stained and ready to be processed by image analysis. Each sample was placed in a Petri dish, and by using a needle the oocytes were gently separated. A photograph of the sample was taken and processed using Image J software. The oocytes diameter was measured as the average of the major and minor axes, and those oocytes larger than 125 µm present in the samples were counted and the size noted. The mean diameter of the most advanced vitellogenic oocyte in females in the advanced vitellogenic state, estimated from the largest 100 oocytes, was also measured for assessment of its seasonal variation.

In an effort to assess the seasonal variation in the percentage of different oocyte stages present in the ovary during the spawning season, the diameter range for each oocyte stage was estimated in histological sections and applied to whole-mounts analyzed by image analysis. Using histological sections the diameters (i.e. the average of major and minor axes) of 75 oocytes per oocyte stage were measured. The limits for each stage were established by applying the 99% confidence interval. The mean value of the corresponding limits was used to segregate the oocyte stages.

Statistical analysis

An ANOVA test was applied to investigate the differences in the proportion of oocytes in the cortical alveolar stage in females in the spawning capable phase by month. Simple regression analysis was used to establish the existing relationship between the mean diameter of advanced vitellogenic oocytes and month.

Results

Oocyte developmental stages

By analyzing the histological sections, the females were classified in the corresponding reproductive phases (Table 6.1). In the mature phase the ovary appears to be a random mixture of oocytes in all stages of development with no dominant population present. Therefore, the skipjack´s oocyte development is defined as asynchronous.

Table 6.1. Total number of fish in each reproductive phase sampled randomly by month in the Western Indian Ocean. Histological markers were used to define each phase. In the immature ovaries, PG oocytes were present. The ovaries in the mature phase were classified according to the most advanced oocyte stage present in the gonad (i.e. CA, Vtg1, Vtg2, Vtg3 and GVM). Females in the regressing phase showed ≥50% of the oocytes at the α-atretic stage.

Month	**Immature**	**Mature**						**Total N**
		Developing		**Spawning Capable**			**Regressing**	
	PG	**CA**	**Vtg1**	**Vtg2**	**Vtg3**	**GVM**		
January, 2009			2	2	15		1	20
February, 2009	1	1		17	29	21	14	83
March, 2009	3	13	3	38	97	2	28	184
April, 2010	38	39	8	19	86	19	12	221
May, 2010	14	11	3	9	18	5	6	66
June, 2009	3	3		11	17		3	37
July, 2009	2	1	3	13	31	8	4	62
Total n	61	68	19	109	293	55	68	673

The oocytes develop from the primary oocyte growth stage, PG, to the secondary growth stage, which is subdivided into various stages (i.e. cortical alveolar CA, and primary Vtg1, secondary Vtg2, and tertiary vitellogenesis stages Vtg3), to progress throughout the oocyte maturation (germinal vesicle migration, GVM, and final hydration) (Table 6.2; Fig. 6.2). The yolked oocytes are hydrated, ovulated and spawned in several batches. In this process the oocytes are released from their follicles (i.e. granulosa and thecal layers) into the lumen.

Table 6.2. Summary of the secondary oocyte growth stages defined for *Katsuwonus pelamis.* A morphological description of each stage and the range size of the oocytes in µm are given. The diameters were estimated by measuring oocytes in histological slides

Oocyte development stage	**Description**	**Diameter (µm)**
Cortical alveoli	This stage is the primary indicator of the onset of the oocyte development for the breeding season. It is characterized by the appearance of small spherical vesicles, i.e. cortical alveoli, in the periphery of the cytoplasm. These increase in size and number as the oocyte develops. Oil vacuoles begin to accumulate in the cytoplasm. The chorion (i.e. zona radiata) also appears in this stage.	125-220
Vitellogenic stage	This stage lasts from the appearance of yolk vesicles in the cytoplasm to their fusion during the final maturation. It is subdivided into three sub stages, taking into account the accumulation of the granules containing the yolk proteins (i.e. vitelogenin) within the oocyte´s cytoplasmic area.	
Vtg1	Oil droplets occupy more cytoplasmic area than yolk granules.	220-330
Vtg2	Oil droplets occupy a similar cytoplasmic area to yolk granules.	330-420
Vtg3	Oil droplets occupy a smaller cytoplasmic area than yolk granules.	420-480
Maturation		
GVM	The germinal vesicle starts to migrate towards the animal pole where the micropyle is situated, while the lipoid vesicles coalesce in a unique oil globule.	480-540
GVBD	Nuclear migration continues while the yolk granules start to fuse.	540-650

At the end of the spawning cycle, the oocytes in the secondary growth stages suffer a resorption process called atresia, which is subdivided into different stages. In the initial stage of the atresia (α- atresia) the entire oocyte including the yolk (if present) is reabsorbed by the hypertrophying granulosa cells of the follicle (Fig. 6.2). Subsequently, major degeneration of the granulosa and thecal cells occurs, reducing the size of the follicle (β-atresia).

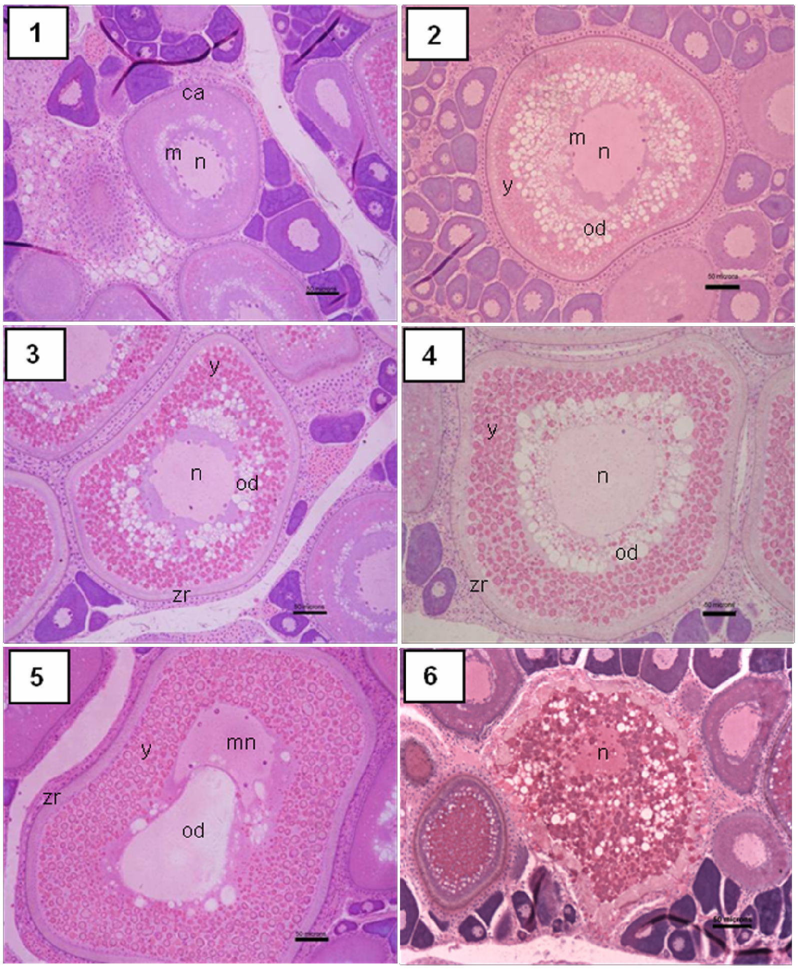

Fig. 6.2. Stages of oocyte development in the *Katsuwonus pelamis.* (1) Oocytes in Cortical alveolar CA stage; 20x. (2) Oocytes in primary vitellogenic stage-Vtg1 stage; 20x. (3) Secondary vitellogenic stage-Vtg2 oocyte; 20x. (4) Tertiary vitellogenic stage-Vtg3 oocyte; 20x. (5) germinal vesicle migration stage-GVM oocyte; 20x; (6) Oocytes in alpha-atresia stage; 20x. Bar on the image is equivalent to 50 µm. n: nucleus; m: nucleolus; ca: cortical alveoli; zr: zona radiata; y: yolk granules; od: oil droplets; mn: migratory nucleus.

Oocyte diameter size distribution:

Skipjack ovaries show a continuous oocyte diameter distribution throughout the different phases of gonad development. A gap in diameter distribution cannot be observed between the primary growth stage and those oocytes entering the secondary growth stage at 125 µm. In the ovaries of fish in the developing and spawning capable phase the oocytes gradually increase in size along with the ovarian development (Fig. 6.3). At the early developing phase the ovary contains small oocytes in the primary growth and cortical alveolar stage (125-220 µm), distributed uniformly in number. During the subsequent vitellogenesis process, the size of the oocytes increases from a minimum of 220 µm in Vtg1 to a maximum of 480 µm in Vtg3. At this development phase a group of oocytes within the secondary growth stage is separated from the adjacent group of smaller oocytes, which will develop to form the next batch. In this sense, a gap develops in the frequency distribution of oocytes at 375-425 μm (Fig. 6.3). In females in the actively spawning sub-phase, this gap will separate the spawning batch from the standing stock of oocytes. The oocytes in the batch continue increasing in size (from GVM to the hydration stage) while the remaining oocytes stay in the vitellogenic stage. This group of oocytes will be spawned in the subsequent batch, while the oocytes from the reserve will continue to increase in size to be spawned in successive batches.

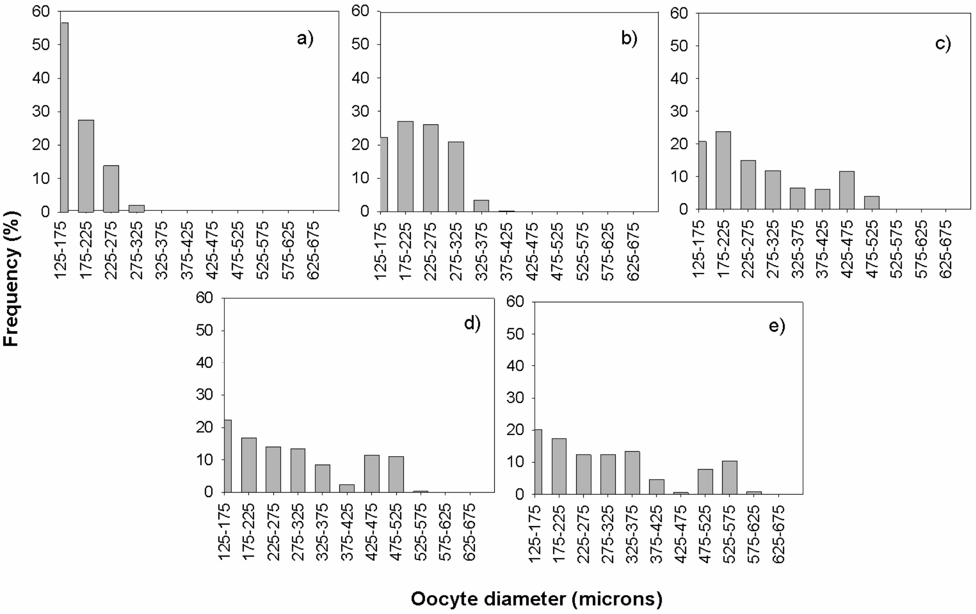

Fig. 6.3 Frequency distribution of oocytes (whole-mount) by 50 μm diameter size at different reproductive phases: early developing phase with cortical alveoli (a) and Vtg1 (b); and spawning capable phase with Vtg2 (c), Vtg3 (d) and GVM (e) as the most advanced oocyte type. Each panel corresponds to an individual fish.

It has been observed that in ovaries in the migratory nucleus phase, the oocytes in Vtg3 stage are scarce (Fig. 6.4), implying that the spawning batch begins to separate in size in the Vtg2 stage and it develops to be spawned from the Vtg3 stage onwards.

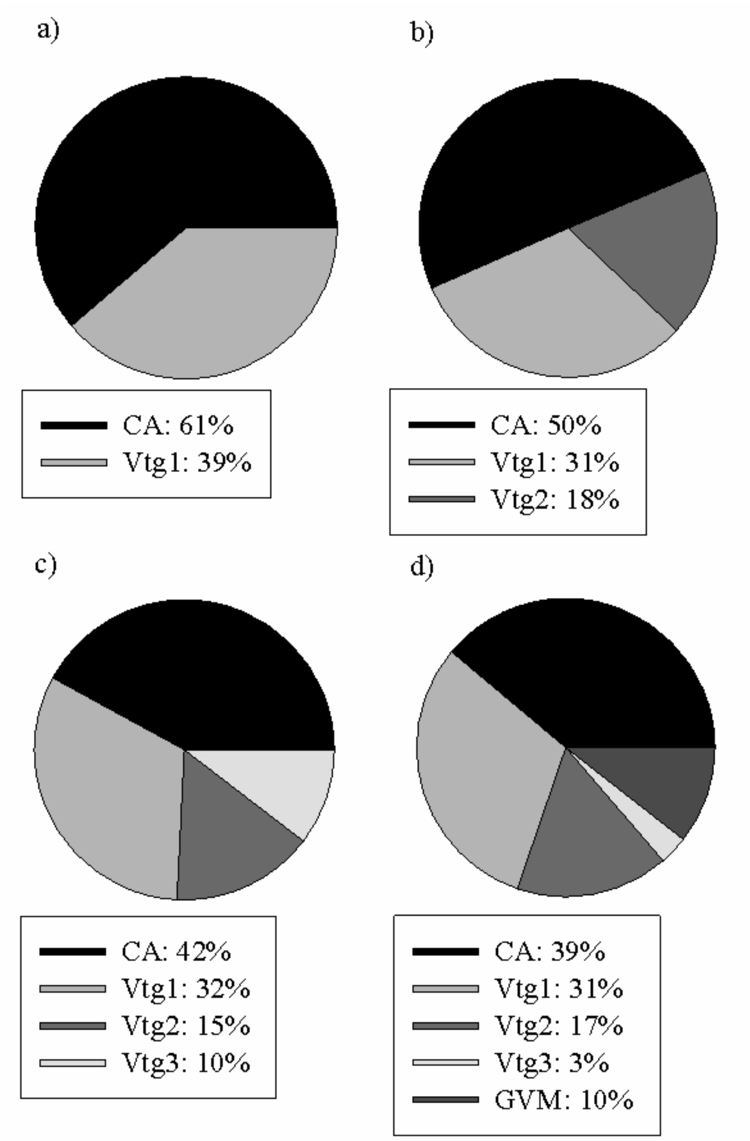

Fig. 6.4. Average percentage of oocyte stages in females in the developing and spawning capable phase with Vtg1 (a; n=15), Vtg2 (b; n=18), Vtg3 (c; n=35) and GVM (d; n=19) oocytes as the most advanced oocyte type present in the ovary. Interval size of oocyte stages was estimated by histology and applied to whole-mount. The values include the whole sampling period.

The described oocyte growth pattern was observed during all the sampling periods, and no seasonal variation in either developing ovaries or in those in the spawning capable phase was observed (Fig. 6.5).

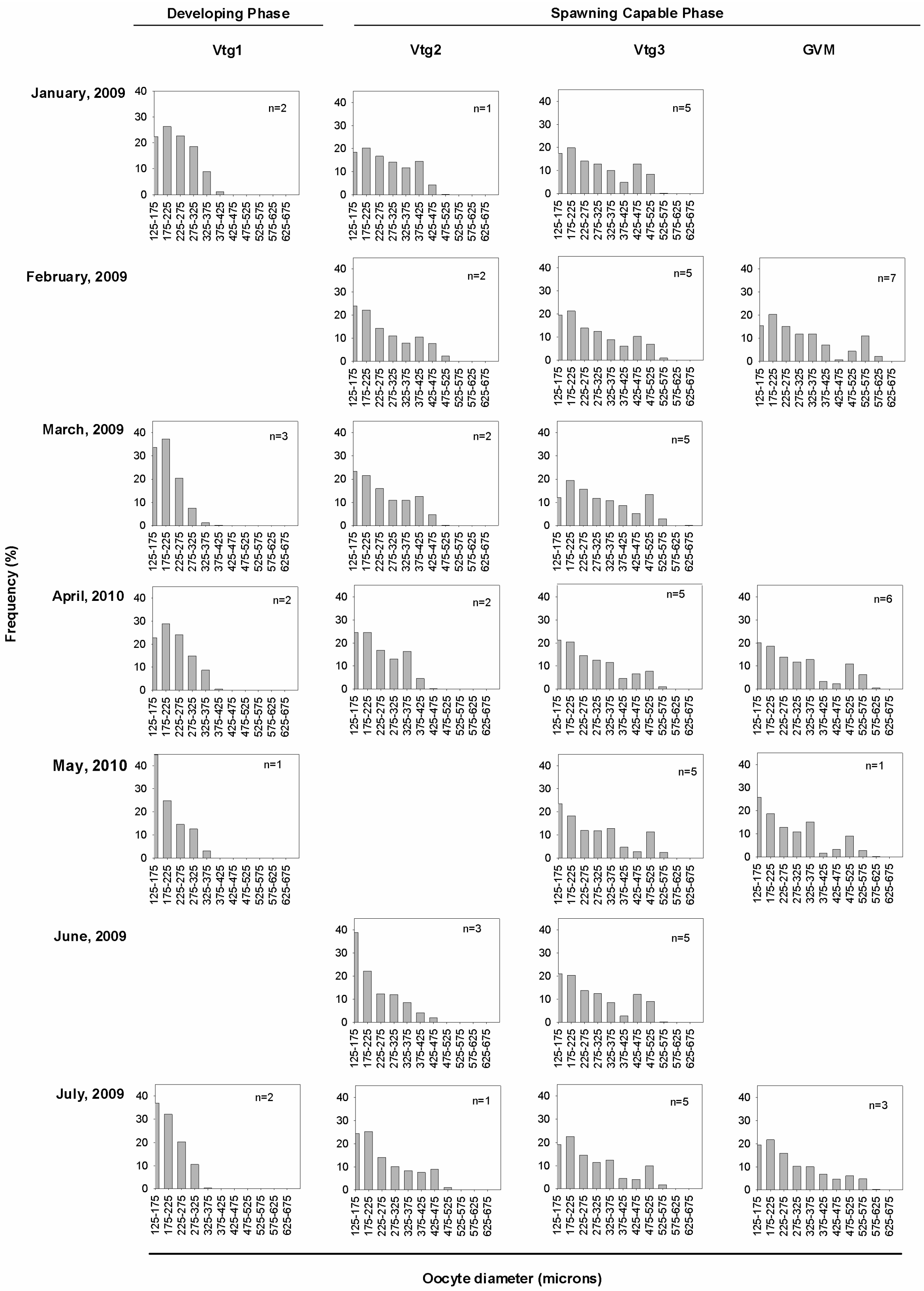

Fig. 6.5. Monthly change in frequency distribution of oocytes (whole-mounts) by 50 μm diameter size classes in ovaries in different reproductive phases.

Seasonal variation in the relative number of the oocyte stages in females in the spawning capable phase

The percentage of different oocyte stages in ovaries of females in the spawning capable phase, having Vtg3 as the most advanced oocyte stage present in the ovary, was studied by month (Fig. 6.6). Overall, the frequency distribution of oocytes did not show a significant variation during the sampling period. When considering the cortical alveolar stage alone (125- 220 µm), it corresponded during the sampling period to a mean of 41.9±3.6% of the total pool of oocytes, going from a minimum of 36.6±4% (i.e. March, 2009) to a maximum of 46.6±6.9% (i.e May, 2010). When comparing the ratio of the relative number of oocytes in the CA stage and the total pool by month, it could be seen that there were no significant differences among months (F-ratio=1.8; p=0.14). The relative number of total vitellogenic oocytes showed a mean of 58.1±3.6% of the total pool of oocytes in the ovary during the sampling period, going from 53.4±11.6 in May to 63.5±9.3 in March.

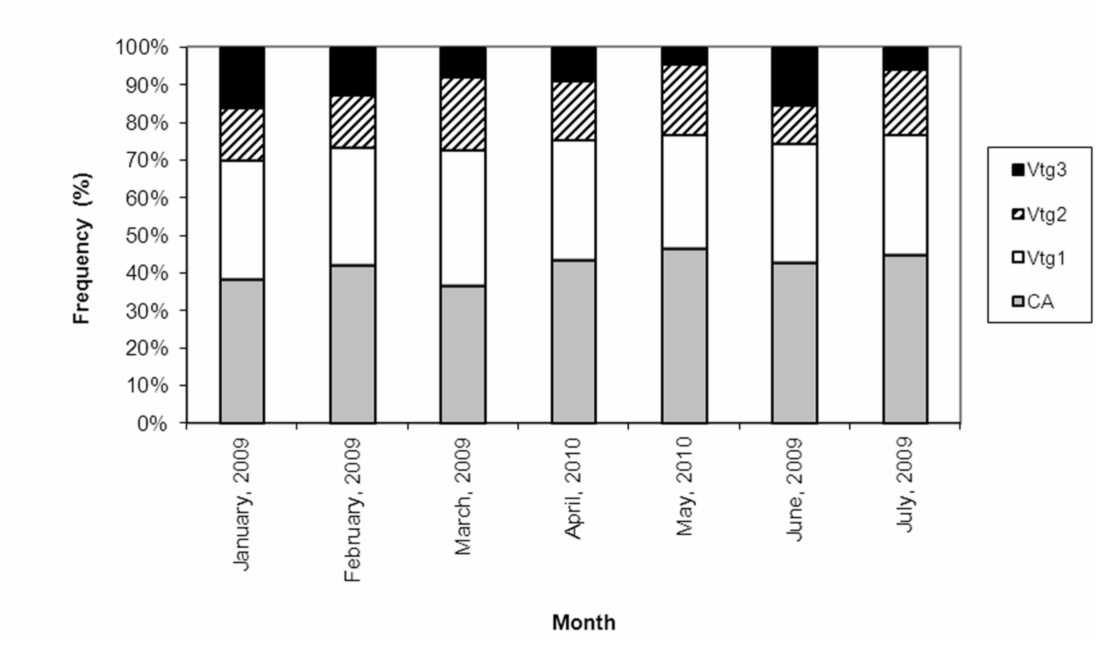

Fig. 6.6. Seasonal variation in the percentage of oocyte stages in females in the spawning capable phase with Vtg3 as the most advanced oocyte stage present in the ovary. Five individuals were analyzed per month. The interval size of the oocyte stages was estimated by histology and applied to whole-mount samples.

Seasonal variation in the mean diameter of oocytes in the most advanced vitellogenic stage in females in the spawning capable phase

The mean diameter of the most advanced vitellogenic oocytes varied within a range of 38.9 µm between months. There was no statistically significant difference in the mean diameter among the months (ANOVA, F-ratio = 1.00264, p = 0.4432). As the mean diameter was constant during the sampling period in 2009, the model as fitted only explained 0.006% of the variability. Therefore, there was no significant relationship with the seasonal component, at least at the 95% confidence interval.

Incidence of atresia

In mature individuals the prevalence of atresia (i.e. percentage of females showing any signs of atresia) did not exceed 40% over the sampling period (Table 6.3). During January the frequency of individuals showing any sign of atresia was the lowest, 10%. This level increased sharply during February and maximum values were reached in March (37%), when 15.5% of the females had entered the regressing phase (i.e. atretic state 2 and 3, where ≥50 % of the yolk oocytes in the ovary show signs of atresia). This fact was related to the end of the first peak of the spawning of the population. During April and May, the prevalence of atresia decreased steadily and the number of females in the regressing phase was reduced. During these months we found more individuals in the early developing phase (having gametes at CA stage) (Table 6.1) indicating the initiation of the cycle, which will probably end with the spawning of gametes in the forthcoming spawning season (during June and July). None of the fish was in the regressing phase when entering the second peak of the reproductive season (i.e. June and July).

Table 6.3. Monthly change in the number of mature females (n=612) in each atretic state and the corresponding prevalence of atresia by month. Also, the percentage of females having ≥50% of the yolked oocyte in atretic stage (i.e. females in regressing phase) is shown.

Month	Jan.-09	Feb.-09	Mar.-09	Apr.-10	May-10	June-09	July-09	Total N
N° of fish	20	82	181	183	52	34	60	612

Atretic state	Number of fish							
0	18	54	114	145	39	30	53	453
1	1	14	39	26	7	1	3	91
2	1	9	20	10	5	3	4	52
3		5	8	2	1			16

Prevalence	10	34,1	37,0	20,8	25,0	11,8	11,7
Regressing	5	17,1	15,5	6,6	11,5	8,8	6,7

Discussion

In this study, four criteria were analyzed in order to describe the oogenesis pattern of the skipjack and classify the type of fecundity as determinate or indeterminate (Hunter *et al.*, 1992; Greer Walker et al., 1994; Murua and Saborido-Rey, 2003). Based on the histological sections and the oocyte size frequency distribution (observed in whole-mount samples), we describe the ovarian development organization of the skipjack as asynchronous, and we consider the fecundity to be indeterminate.

Regarding the oocyte size frequency distribution, no distinct hiatus was observed between the primary and secondary oocytes growth stages (Hunter *et al.*, 1985; Lowerre-Barbieri *et al.,* 2011*a*). This pattern was observed in all the ovarian developmental phases and during all the sampling periods. Although this is not an essential criterion for defining the fecundity type, it may indicate that the fecundity is indeterminate with a protracted spawning period and multiple spawning events (Hunter and Macewicz, 1985*a*; West, 1990; Greer Walker *et al.*, 1994). This oocyte distribution pattern has also been observed by other authors (e.g. Timohina and Romanov, 1996; Cayré and Farrugio, 1986; and Stéquert and Ramcharrun, 1996).

The development of the gap in the oocyte size distribution occurs when the next batch of oocytes is generated. The gap formation usually starts when the oocyte enter the GVM stage and it is completed at hydration (Hunter *et al.,* 1985); however, in the case of skipjack gap formation was identified in an earlier ovarian developmental phase (i.e. the tertiary vitellogenic stage). In ovaries at the spawning capable phase, while oocytes were in the secondary yolked stage the next batch of oocytes started to separate from the adjacent population of smaller oocytes, while at the tertiary yolked stage the separation was more evident. The gap was observed when the oocyte diameter was around 375-425µm. This range is similar to the one identified in other studies in the Indian Ocean and others (Cayré and Farrugio, 1986; Stéquert and Ramcharrun, 1995; Stéquert and Ramcharrun, 1996; Timohina and Romanov, 1996). The same pattern was observed in other species (i.e. *L. litulon, S. pilchardus sardina*). In previous cases it was attributed to a low rate of clutch recruitment and a large inter-spawning interval (Yoneda *et al.,* 2001; Ganias *et al.*, 2004); however, in the case of skipjack, previous data suggest a short spawning interval, very close to daily spawning, in the Pacific region (i.e. 1.2 days between batches) (Hunter *et al.,* 1986). Therefore, the early formation of the batch cannot follow the same mechanism as those seen in other species in the literature. Due to the high metabolism of the skipjack, they are capable of adopting a fast reproductive cycle (Hunter *et al.*, 1986). As long as the physiological conditions are favourable the spawning capable ovaries maintain a high rate of spawning, and as soon as they are unable to maintain this investment, the generalized atresia or resorption process occurs to finish the individual spawning season. The transition from spawning to regressing phase and vice-versa seem to be a very quick process in which the fish do not invest much energy. However, the cost of reproduction will be high due to the long spawning season and the high spawning rate. The cost of a single spawning is estimated to be 2% of the body weight (Hunter *et al.*, 1986).

Based on the literature, the spawning season of the skipjack takes place all year around with periods of more intensive reproductive activity during the North-east monsoon (i.e. November to March) and South-west monsoon (i.e. June to July) (Stéquert and Ramcharrun 1996; Timohina and Romanov, 1996). Our results, covering different reproductive activity periods and reproductive phases, showed that the oocyte distribution in gonads did not show any difference among months in any of the ovarian developmental phases. In females at the spawning capable phase, the percentage of oocytes in the cortical alveolar and vitellogenic stages remained constant during the whole sampling period. These facts provide evidence of a continuous recruitment of oocytes into the standing stock of developing oocytes, a process only occurring in indeterminate spawners (Hunter *et al*., 1985; Greer Walker *et al*., 1994; Murua and Saborido-Rey, 2003). The lowest number of oocytes in the vitellogenic stage during May is related to the fact that this is a less intense period of sexual activity. The variation in the number of the most advanced vitellogenic oocytes between fish with signs of spawning and those with no signs of spawning could not be evaluated due to the lack of individuals with clearly recognizable POF. This is the result of the quick resorption process of these structures (Hunter *et al.,* 1986) and the difference between fishing and spawning time. The POF resorption time is positively related to water temperature (Kurita *et al.,* 2011), which is considered high in the spawning habitat of skipjack (around 24-30 º C). On the other hand, there is an elapsed time between the fishing and spawning events, which are known to occur usually at sunrise and at night, respectively (Hunter *et al*., 1986).

Depending on the fecundity style adopted by the species, the mean size of the most advanced oocytes can change during the spawning season. In determinate spawners the mean diameter of the advanced vitellogenic oocytes increases during the spawning season due to a lack of replenishment of the spawned oocytes from the newly recruited ones (Hunter *et al.,* 1986; Greer Walker *et al.,* 1994; Murua and Saborido-Rey, 2003). In this study, the size of the most advanced vitellogenic oocytes was defined as constant during the period of 2009. This indicates that newly yolked oocytes are being recruited during this period. This supports the idea of indeterminate fecundity, as the recruitment of oocytes continues after the onset of the spawning.

In teleost fish a number of factors have been identified as causing follicular atresia (e.g. temperature changes, starvation, stress) (Guraya, 1986). The atresia is described as a highly down-regulation process for the maintenance of ovarian homeostasis and is a trade-off between the number and size of the oocytes and the available energy allocation resources (Kjesbu, 2009). In indeterminate fish it is usual to find a generalized intensity of atresia at the end of the spawning season. This occurs in order to resorb the surplus production of oocytes (Guraya, 1994), while a low incidence is observed throughout the spawning season (Hunter and Macewicz, 1985; Hunter *et al.,* 1986; Greer Walker *et al*., 1994; Murua and Saborido, 2003; Murua *et al*., 2003). For the skipjack a generalized resorption of vitellogenic oocytes was observed during February and March, coinciding with the end of the season of active reproductive activity. This evidence is the fourth criterion supporting the idea of the indeterminate fecundity of the skipjack.

Conclusions

Based on the findings, it can be concluded that the skipjack in the Western Indian Ocean show an asynchronous oocyte growth and indeterminate fecundity type. Although previous studies also support the idea of the asynchronous oocyte growth and the indeterminate fecundity type of the skipjack (Raju, 1964; Batts, 1972; Stéquert, 1976; Matsumoto *et al.,* 1984; Goldberg and Au 1986; Cayré and Farrugio, 1986; Stéquert and Ramcharrum, 1995; Stéquert and Ramcharrum, 1996), this is the first research in which the reproductive strategy is studied in detail based on several accepted criteria (Hunter *et al*., 1992; Greer Walker *et al.*, 1994; Murua and Saborido-Rey, 2003). In summary, in the ovaries of females in the spawning capable phase (i) oocytes of different sizes and developmental stages are present; (ii) the individuals show a broad oocyte size frequency distribution without any gap formation between the primary and secondary growth stages because the recruitment of oocyte into vitellogenesis is detected all year around, at least at the population level; (iii) there is a continuous replenishment of new oocytes from the cortical alveolar stage to the pool of vitellogenic oocytes throughout the year; and (iv) when reaching the end of the high reproductive period (i.e. February and March) the number of females showing atresia increases, with 100% of the oocytes in an atretic stage.

7. Chapter II

Sex ratio, reproductive timing and reproductive capacity of the skipjack tuna Katsuwonus pelamis in the Western Indian Ocean

Introduction

Skipjack tuna *Katsuwonus pelamis* (L. 1758) is a cosmopolitan species widely distributed throughout the world's tropical and subtropical oceans. Its geographical limits are 55º-60ºN and 45º-50ºS, and it is more abundant in the equatorial region all year round (Matsumoto *et al.*, 1984). It is an economically valuable species worldwide (FAO, 2010), and in the Indian Ocean it makes up around half of the total tuna catches (IOTC, 2011).

Several recent works have studied the biology of skipjack worldwide, including its growth (Gaertner *et al.*, 2008), diet (Jaquemet *et al.*, 2011), bioenergetics (Essington, 2003), recruitment (Andrade and Santos, 2004), habitat (Mugo *et al.*, 2010), population structure (Dammannagoda *et al.,* 2011), and reproduction (Schaefer, 2001*a*). Previous works focusing on its reproductive biology have described the fecundity type (Grande *et al.*, 2012), reproductive timing in terms of size at maturity and spawning season (Stéquert and Ramcharrun, 1996; Timohina and Romanov, 1996; Stéquert *et al.*, 2001). The variation in the reproductive outputs, i.e. fecundity, has been assessed in the Western Indian Ocean (Stéquert and Ramcharrun, 1995; Timohina and Romanov, 1996) and other basins (Matsumoto *et al.*, 1984; Hunter *et al.*, 1986; Cayré and Farrugio, 1986; Batalyants, 1989; Schaefer, 2001*a*; Schaefer, 2001*b*). These studies describe the skipjack as having an indeterminate fecundity type (Grande *et al.*, 2012). In the Western Indian Ocean the spawning season is long and takes place year around with periods of more intensive reproductive activity during the North-east monsoon (i.e., November to March) and South-west monsoon seasons (i.e., June to July) (Stéquert *et al.*, 2001). Previous data suggest a short spawning interval in the Pacific region (i.e., 1.2 days between batches) (Hunter *et al.*, 1986).

Although there is evidence that changes in fish condition can alter the productivity of fish populations (Marshall *et al.*, 1999; Marshall *et al.*, 2003) and have a negative impact on fish recruitment (Rideout and Tomkiewicz, 2011), there have been no studies on the contribution that the physiological condition makes to skipjack fecundity. Moreover, it is widely known that fish productivity varies temporally and spatially (Lambert, 2008; Kjesbu, 2009; Korta *et al.*, 2010*b*). Previous fecundity estimates in the

Western Indian Ocean go back to the 80s and 90s (Stéquert and Ramcharrun, 1995), but no recent evaluation has been conducted in the area. Therefore, in order to evaluate variations in the population's reproductive capacity effectively, and thus be able to investigate the resilience of skipjack to fishing pressure, it is essential to describe the effect that the physiological condition has on the reproductive outputs and monitor possible changes in the productivity of the species over time (Lambert *et al.,* 2003; Marshall *et al*., 2003; Wright and Tripple, 2009; Lowerre-Barbieri *et al.*, 2001*a*). On the other hand, recent studies of the reproduction dynamics of fish have introduced new standardized terminology and classification criteria for describing the reproductive development of fish and their maturation processes (Brown-Peterson *et al.,* 2011; Lowerre-Barbieri *et al.,* 2011*a*; Lowerre-Barbieri *et al*., 2011*b*). These studies highlight the importance of using this terminology and criteria in new studies in order to facilitate comparative analyses. In addition, they encourage applying new methodological techniques and reviewing the histology criteria to improve the data available and the accuracy of reproductive studies.

In this context, the present study investigates aspects of the reproductive biology of skipjack, including detailed reproductive data based on a histological staging system. This makes it possible to analyse the reproductive timing in terms of sexual maturity (L_{50}) and spawning season while incorporating the new terminology and classification criteria. In addition, we evaluate the reproductive capacity of the species based on batch fecundity estimates and quantifying the effect on ovary growth (gonadosomatic index I_G) of the fish size, physiological condition (hepatosomatic index I_H and Fulton´s condition factor K) and spatiotemporal factor.

Material and Methods

A total of 1269 fish were collected on board a purse-seiner in the Western Indian Ocean, covering the area off the southeast Seychelles, northwest Seychelles, Somalia and the Mozambique Channel (Fig. 7.1). The sampling was carried out in three surveys, which were performed between January and March 2009, June and July 2009, and April and May 2010 (Table 7.1). The sampling covered the different monsoon seasons occurring in the Western Indian Ocean which are characterized by surface wind reversal: north-easterly winds from December to March (i.e., North-east monsoon season NM); south-westerly winds from June to September (i.e., South-west monsoon season SM); and two corresponding inter-monsoon seasons (IM) from April to May and from October to November (Schott and McCreary, 2001).

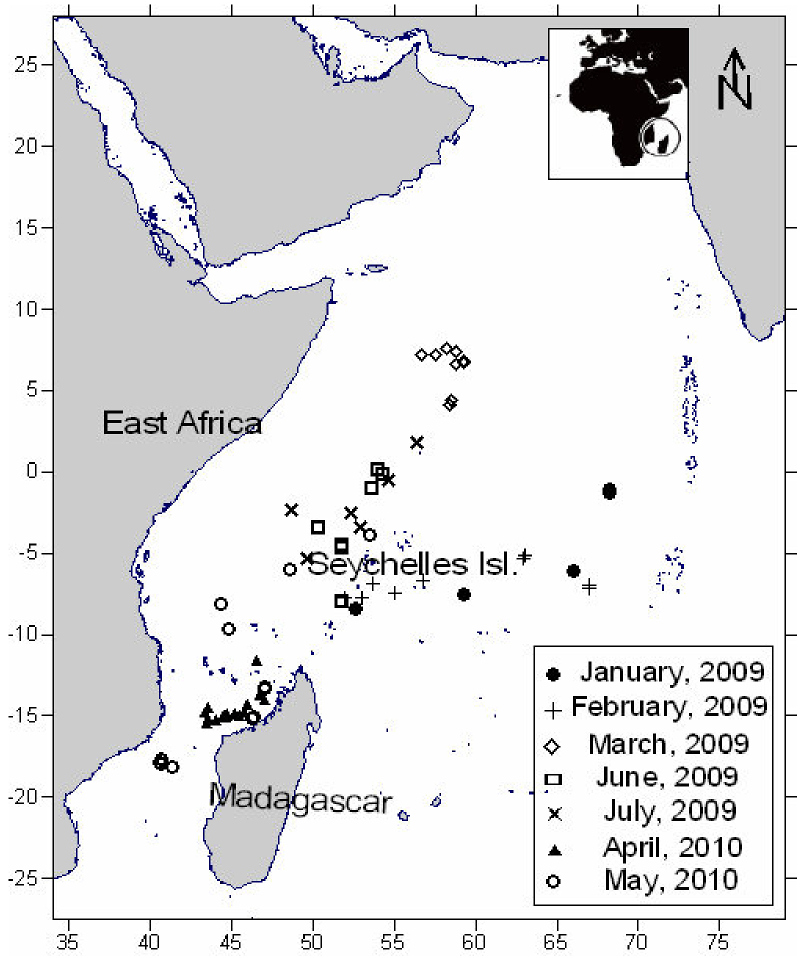

Fig. 7.1 Sampling locations in the study area

Table 7.1 The number of fish (n), the fork length (L_F) range (cm) and mean ± S.D of specimens sampled randomly by month throughout the Western Indian Ocean

Date	n	L_F range	L_F (mean ± S.D)
January 2009	49	37-52	45 ± 2.7
February 2009	193	37-65	47.2 ± 4.4
March 2009	323	33-65	53 ± 5.3
June 2009	79	35-65	49.7 ± 8.1
July 2009	135	33-60	47.1± 5.6
April 2010	380	32-67	44 ± 4
May 2010	110	35-68	47.1 ± 5.6

The fork length L_F (to the nearest 0.5 cm) and total mass M_T (to the nearest g) of the fish were measured on board. The fish were eviscerated and the mass (g) of the liver M_L and gonads M_G was also recorded. The sex of the fish was determined on board whenever possible by examining the gonads. To estimate the sex ratio, the fish caught in the third trip (focused on sampling females), specimens classified as indeterminate and fish smaller than 35 cm and bigger than 65 cm (low number of fish in these size ranges) were not included in the analysis. Chi-square test was used to evaluate the significant deviation from the expected 1:1.

Sub-samples of the posterior right lobule of 673 female ovaries were taken and preserved in a solution of 4% buffered formaldehyde immediately after capture to be used for histological analysis, batch fecundity (F_B) and relative batch fecundity (F_{Brel}) estimation. At the laboratory, cross sections of 5 mm of each ovary were dehydrated by placing them in increasing concentrations of alcohol (i.e., from 70% to 96%) and then soaking them in solutions in which the alcohol concentration was gradually decreased and the resin concentration increased. The samples were then polymerised into resin blocks. The histological sections of 3 to 5 µm were cut using a microtome and were stained with Harris' hematoxylin and eosin. The most advanced oocyte stage seen in the gonad (West, 1990) in microscopic slides was used to classify the ovaries into different reproductive phases following the classification criteria proposed by Brown-Petersen *et al.* (2011) and already applied to skipjack in Grande *et al.* (2012). For a more detailed definition of the oocyte stages and reproductive phases of skipjack used in this work see Grande *et al.* (2012).

The female size at maturity (L_{50}) (i.e., size at which 50% of the population is mature) was estimated by calculating the proportion of histologically mature females by 1-cm size classes. The fish were considered mature when the cortical alveolar CA oocyte stage was present in the gonad (Brown-Peterson *et al.*, 2011; Lowerre-Barbieri *et al.*, 2011*a*). The data were fitted to a logistic equation, where P is the predicted mature proportion, α and β are the coefficients of the logistic equation and L_F is the fork length in cm. A nonlinear regression (the Marquardt method without restrictions; Marquardt, 1963) was used to fit the logistic equation to the data.

$$P = e^{\alpha+\beta L_F}(1+e^{\alpha+\beta L_F})^{-1}$$

Three condition indices were estimated for females: the gonadosomatic index I_G, the hepatosomatic index I_H and the Fulton condition factor K. I_G and I_H were calculated as the gonad or liver mass, M_G and M_L respectively, divided by the gonad free mass $M_{Gfree} = M_T - M_G$ expressed as a percentage, where M_T is the total mass of the fish and M_G is the gonad mass. K was estimated as the M_{Gfree} divided by L_F raised to the third power and expressed as a percentage. In order to determine whether there was a temporal pattern in the spawning activity of the population, differences in the condition indices (I_G, I_H and K) were studied using the nonparametric Kruskal-Wallis test.

$$I_G = (M_G.M_{Gfree}^{-1}).100$$
$$I_H = (M_L.M_{Gfree}^{-1}).100$$
$$K = (M_{Gfree}(L_F^3)^{-1}).100$$

The batch fecundity was determined in 51 females in the actively spawning sub-phase containing oocytes undergoing germinal vesicle migration GVM as the most advanced stage (Brown-Petersen *et al.*, 2011; Grande *et al.*, 2012). To do so the gravimetric method was used and the most developed oocytes (i.e. oocytes in GVM stage) were counted in the whole-mount samples (Hunter and Macewicz, 1985*a*). Three sub-samples of around 0.1 g (± 0.01) (Schaefer, 1987) were immersed in glycerine and the oocytes were counted under a stereomicroscope. F_B was calculated as the weighted mean density of the three sub-samples multiplied by the total mass of the ovaries. F_{Brel} was calculated as the F_B divided by the M_{Gfree} of the fish. The relationships between the F_B, L_F, and M_{Gfree}, and F_{Brel} and L_F were determined by linear regressions. Differences on F_{Brel} among months were evaluated using the nonparametric Kruskal-Wallis test

Finally, Generalized Additive Models (GAM) were used to explore the role of maternal attributes, fishing area and season on the reproductive capacity of the skipjack, expressed as gonadosomatic index (I_G). Based on the oocyte development and recruitment pattern adopted and the early batch formation observed by this species (Grande *et al.*, 2012), fully developed females, defined as those showing Vtg3 and/or GVM at the most advanced oocyte stages (Grande *et al.*, 2012), were selected (n=264). In these females, the relationship between gonadosomatic index (I_G) and hepatosomatic index (I_H), Fulton condition factor (K), fork length (L_F) and spatiotemporal factor (i.e.

categorical variable including four categories: North-east monsoon in Seychelles, NM Sey; North-east monsoon in Somalia, NM So; inter-monsoon in Mozambique Channel, IM Mz; and South-west monsoon in Seychelles, SM Sey) was investigated (Table 7.2).

Table 7.2. Number of females (n), and mean (± SD) of gonadosomatic index (I_G), hepatosomatic index (I_H), fork length (L_F) and Fulton´s condition factor (*K*) by spatiotemporal strata in spawning capable females containing the most developed oocyte in Vtg3 and/or GVM stage.

Spatiotemporal factor	**n**	I_G	I_H	L_F	K
NM Sey	64	3.4±0.8	1.7±0.5	47.2±3.0	2.0±0.1
NM So	89	1.9±0.5	1.3±0.2	54.6±4.9	2.1±0.1
IM Mz	69	2.6±0.7	1.8±0.4	47.0±2.6	1.9±0.1
SM Sey	42	2.5±0.9	1.4±0.3	48.3±5.1	1.9±0.2
Total	264	2.5±0.9	1.5±0.4	49.8±5.0	2.0±0.1

Cleveland dotplots (Cleveland, 1985) and boxplots were used to identify extreme observations. Co-linearity effect between the covariates was explored using pair plots and correlation coefficients. For modelling the I_G a gamma error distribution with “log” link was used (Cubillos and Claramunt, 2009). The selection of predictors and the degree of smoothing was done on the basis of generalized cross-validation (GCV) score. Models were also compared by approximate hypothesis testing by using the F test (Wood, 2006). We used R statistical software and generalized additive models were built using the mgcv package.

Results

Sex ratio

A total of 1269 fish were sampled, of which 702 (55.32%) were females, 505 (39.80%) were males and 62 (4.89%) were indeterminate. The fish ranged from 32 to 68 cm in fork length. The overall sex ratio did not differ significantly from the expected 1:1 (Table 7.3). In addition, the sex ratio estimated for 5-cm size classes was not significantly different from 1:1 (Table 7.3). There were more females than males in all size classes except in the largest size class, in which males dominated. However, the differences were not significant in any of the size classes.

Table 7.3. Sex ratio of *K. pelamis* sampled during the first survey (January to March, 2009) and second survey (June to July, 2009). The L_F is the fork length range in cm, *F* is the number of females and *M* the number of males.

L_F range	*F*	*M*	Total n	*F:M*	Chi-Squared
[35-40)	17	12	29	1:0.71	0.86
[40-45)	53	48	101	1:0.91	0.25
[45-50)	136	121	257	1:0.89	0.88
[50-55)	79	78	157	1:0.99	0.01
[55-60)	109	95	204	1:0.87	0.96
[60-65)	7	11	18	1:1.57	0.89
Total n	401	365	766	1:0.91	1.69

Length at maturity (L_{50})

The fitted logistic model explained 92.6% of the variability of the proportion of mature females by L_F (Fig. 7.2). The estimated length at which 50% of the female population reached maturity was 39.9 cm. 100% of the females were mature at 56 cm.

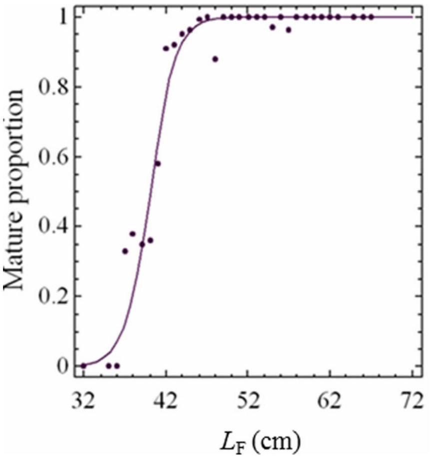

Fig. 7.2. Logistic regression model for the estimated proportion of mature *K. pelamis* females as a function of the fork length L_F (cm), using histological staging system (n=673)

Seasonal variation in occurrence of various reproductive phases

During the sampling period there was a dominance of mature females in the spawning capable phase and actively spawning sub-phase, making up 77.3%, of which 76.1% were close to spawning (i.e., in the Vtg3 and GVM stages). A seasonal pattern was observed in the occurrence of various reproductive phases (Fig. 7.3). The number of fish in the spawning capable phase was highest during January and February (NM) (85%), while in the actively spawning sub-phase the number of females was the highest in February (25.6%). The percentage of the mature population in the spawning capable phase decreased steadily from January (NM) to May (IM) (51.9%), then increased again during June (82.4%) and July (73.3%) (SM). For the fish in the regressing phase (i.e., ≥ 50% of the yolk oocytes show signs of atresia), the highest values were observed in February (17.1%) and March (15.5%) (NM) and the lowest values during January (NM) (5%) and July (SM) (6.7%). No specimens were found in the hydration stage.

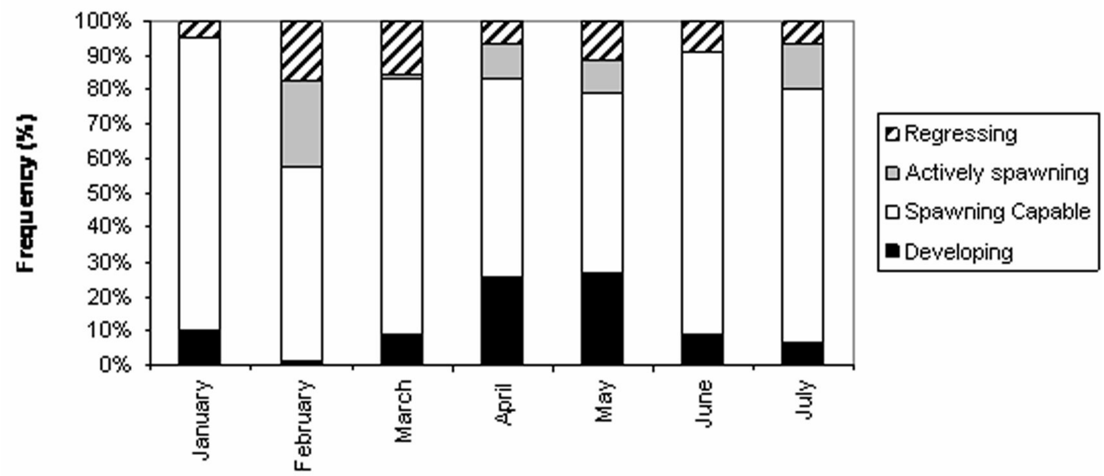

Fig. 7.3. The percentage of mature females classified by the histological staging system in each reproductive phase (developing, spawning capable, actively spawning and regressing phase) by month during 2009 and 2010 in the Western Indian Ocean

Seasonal variation of the condition indices

The I_G data (Fig. 7.4*a*) supported the seasonal reproductive pattern observed with the histological information (Fig. 7.3). During the sampling period, I_G varied between 0.1 and 5.2 with a mean of 1.9 ± 1.0, which corresponds to the vitellogenic stage. This suggests that a high proportion of females were in the spawning capable phase from January to July during 2009 and 2010 all around the Western Indian Ocean. Significant differences in I_G were observed among months ($H_{(6,\ n=533)} = 100.2$, $p < 0.05$). A marked peak indicative of high reproductive activity was detected during January (2.5 ± 0.9, n = 20) and February (2.9 ± 1.1, n = 80), which could be related to the NM. The I_G then decreased to the lowest values in May (1.3 ± 0.9, N = 61), during the IM. It increased again during June (1.6 ± 0.8, n = 31) and July (2.1 ± 1.1, n = 53), i.e., the SM, indicating that this was a recovery period in the reproductive activity. However, when the I_G was analysed by size classes (Fig. 7.4*b*) it was observed that the small fish (i.e., < 45 cm) were the most influenced by this above-described seasonal pattern. They show significant differences among months ($H_{(6,\ n=127)} = 40.31$, $p < 0.05$). In this size range the highest values were observed in January and February, i.e., the NM (2.2 ± 1.0 and 2.2 ± 1.1 respectively) and the lowest during April and May, i.e., the IM (0.6 ± 0.4 and 0.6 ± 0.6 respectively). Overall, the highest I_G (2.4 ± 0.5, equivalent to the Vtg3 stage) was observed in the specimens ranging from 45 to 50 cm. The I_G of these fish was significantly different among months ($H_{(6,\ n=242)} = 40.7$, $p < 0.05$), and the highest values were reached in February NM (3.1±1.1) and the lowest values in March NM (1.5±0.7). In the largest specimens (> 50 cm) the overall I_G was 1.9 ± 0.7, which corresponds to the Vtg2 stage. In this size range significant differences were also observed among months ($H_{(5,\ n=210)} = 32.08$, $p < 0.05$). The highest values corresponded to February NM (2.5 ± 0.8) and April IM (2.6 ± 0.6); and the lowest values to May IM (1.1 ± 0.6).

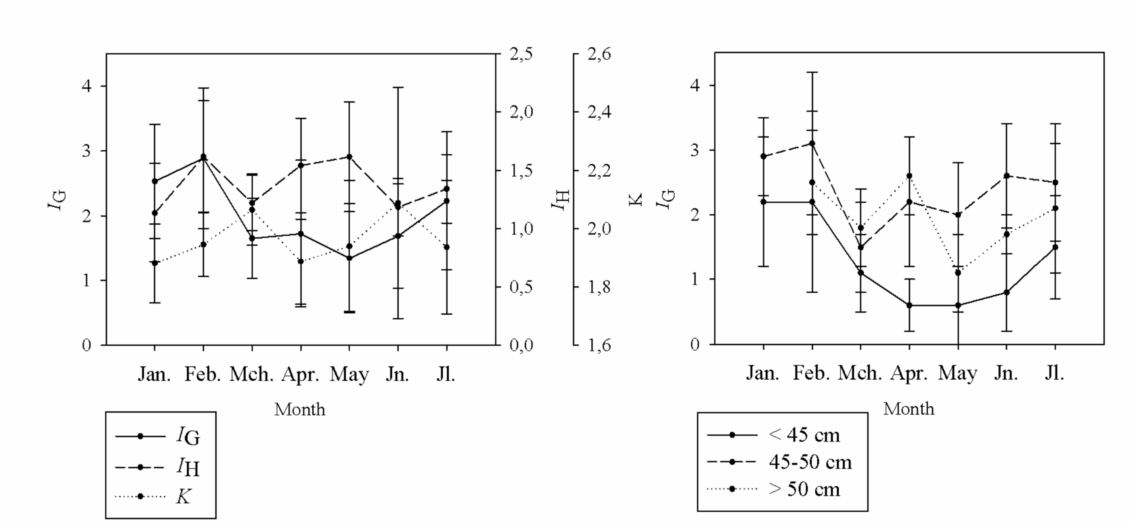

Fig. 7.4. Monthly variation (mean ± SD) (a) in the gonado-somatic index I_G, hepato-somatic index I_H and condition factor *K* during the sampling period; and (b) in the gonado-somatic index I_G by size class (i.e., < 45cm; 45-50 cm; > 50 cm) during the sampling period

The I_H also showed significant monthly variation ($H_{(6,\ n\ =\ 533)} = 94.2$, $p < 0.05$) during the study period (Fig. 7.4*a*). It fluctuated between 0.3 and 3.1. The highest values were observed during February in the NM (1.6 ± 0.5, n = 80) and May during the IM (1.6 ± 0.5, n = 61). This second peak in the I_H does not match the second peak in the I_G, but it is related to the high I_G values of large specimens (Fig. 7.4*b*). This suggests that females are recruited into the spawning population continuously. Minimum values were found in January and March during the NM (1.1 ± 0.4, n = 20 and 1.2 ± 0.2, n = 165 respectively). Finally, significant seasonal variations were also observed in *K* ($H_{(6,\ n\ =\ 533)} = 119.9$, $p < 0.05$) (Fig. 7.4*a*), which ranged between 1.65 and 2.37. During the sampling period, *K* showed the inverse trend to I_H. Two increasing trends were observed: one from January (1.9 ± 0.1, n = 20) to March (2.1 ± 0.1, n = 165) during the NM; and the other from April in the IM (1.9 ± 0.1, N = 123) to June in the SM (1.9 ± 1.2, n = 31). Large variations were observed in the three condition indices within the months, indicating that spawning activity is asynchronous at the population level.

Batch Fecundity

F_B ranged from 100,828 oocytes to 627,325 oocytes. F_{Brel} ranged from 48 to 299 eggs g^{-1} of fish with a mean of 140 ± 64 eggs g^{-1}. High variability was observed when F_B was plotted against L_F and M_{Gfree}; and F_{Brel} against L_F (Fig. 7.5). Although the relationships between F_B and L_F and M_{Gfree}, were statistically significant ($r^2 = 0.1$, F = 5.1, p = 0.03; $r^2 = 0.2$, F = 9.1, p= 0.004; respectively), the variability in F_B explained by the models was low. The highest values were obtained at intermediate size classes, ranging from about 45 to 49 cm and around 2 kg in gonad free weight. On the other hand, the relationship between F_{Brel} and L_F was not significant (p=0.744).

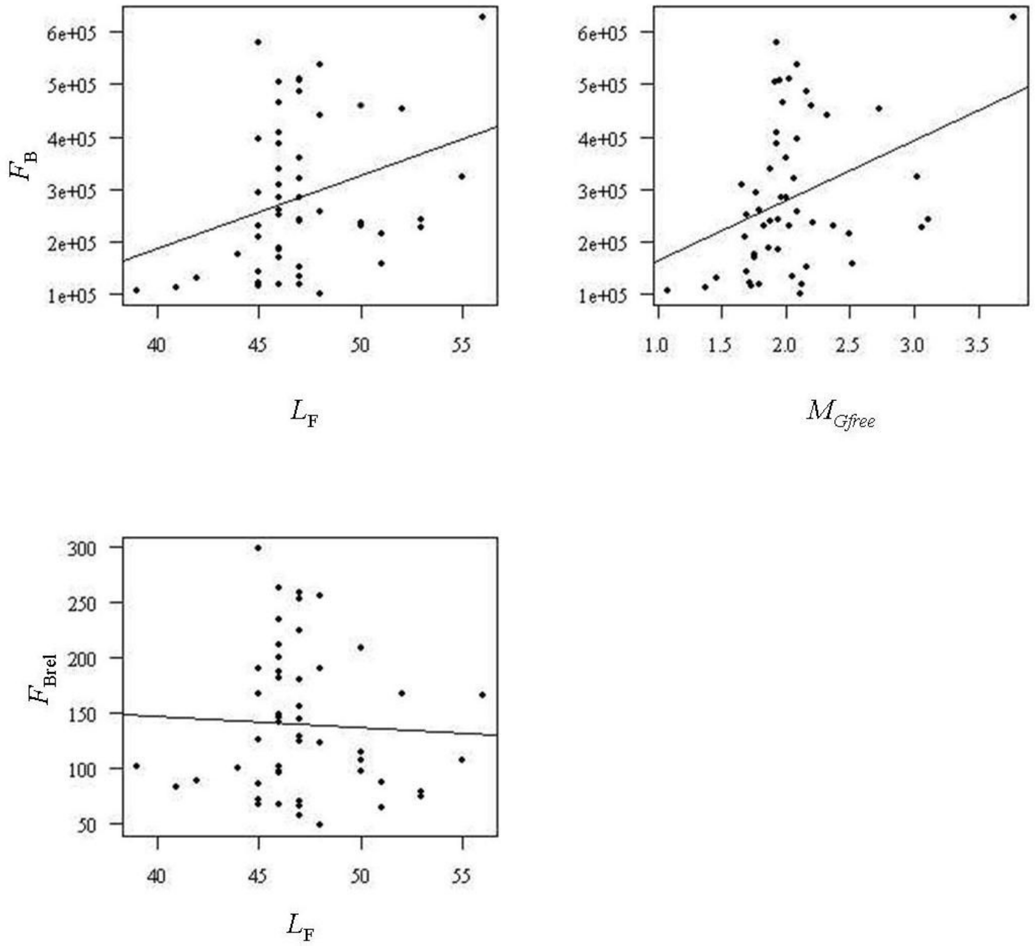

Fig. 7.5. The batch fecundity (F_B) and relative batch fecundity (F_{Brel}) of 51 females in the actively spawning sub-phase in relation to the fork length L_F (cm) and gonad free weight M_{Gfree} (kg)

The F_{Brel} estimates showed a seasonal pattern similar to the seasonal trend observed in I_G. F_{Brel} was highest in February during the NM (169.6 ± 13.3 eggs g^{-1}, n = 21), decreased during April (118.5 ± 15.2 eggs g^{-1}, n = 16) and May (100.2 ± 27.2 eggs g^{-1}, n = 5) during the IM, and then recovered again in July (129.3 ± 21.5 eggs g^{-1}, n = 8) during the SM. However, the differences among months (i.e., February, April, May and July) were not significant ($H_{(3,\, n = 51)} = 5.9$, $p = 0.118$).

Assesment of the variation in the reproductive capacity

The selected GAM model for I_G is summarized in Table 7.4. The model explained 58.7% of the deviance and the CGV score was 0.0618. The smooth function of K was not significant (Table 7.4). However, when comparing models with and without K, CGV score was the lowest in the former and the analysis of deviance using F test show that this predictor could not be rejected ($p < 0.05$) from the model.

Table 7.4. Analysis of variance of Generalized Additive Models applied to study change in the gonadosomatic index (I_G) as a function of hepatosomatic index (I_H), fork length (L_F), condtion index (K), and spatiotemporal factor on spawning capable females containing oocytes in Vtg3 and/or GVM stage (n=264). A gamma distribution with log link was assumed for I_G.

Smooth terms	**Estimated d.f.**	**Non parametric F**	**p-value**
s(I_H)	4.732	9.214	$8.02*10^{-16}$
s(L_F)	7.822	2.952	0.0028
s(K)	3.293	1.552	0.186
Parametric term	**d.f**	**F**	**p-value**
spatiotemporal factor	4	957.7	$<2*10^{-16}$

The GAM plots (Fig. 7.6) show the smooth functions of the predictor variables of the final model. I_H is positively related to I_G. I_G increases with L_F in fish ranging from 39 cm to a maximum at about 47 cm. From that size on, I_G remained at relative lower levels while fish size increased until another peak was observed in large fishes. However, few data points were available at large sizes. The partial effect of K on I_G was negative, with highest K corresponding to lowest I_G. However this term was not significant (p = 0.19, and 0 was included inside the confidence interval all along the K distribution range). As shown in Table 7.4, the spatiotemporal factor resulted to be significant. I_G was the highest in NM Sey followed by SM Sey. I_G was the lowest in IM Mz and NM So. However, as observed in Table 7.2 the I_G reached the second highest value in IM Mz, surely due to an effect of I_H and/or L_F.

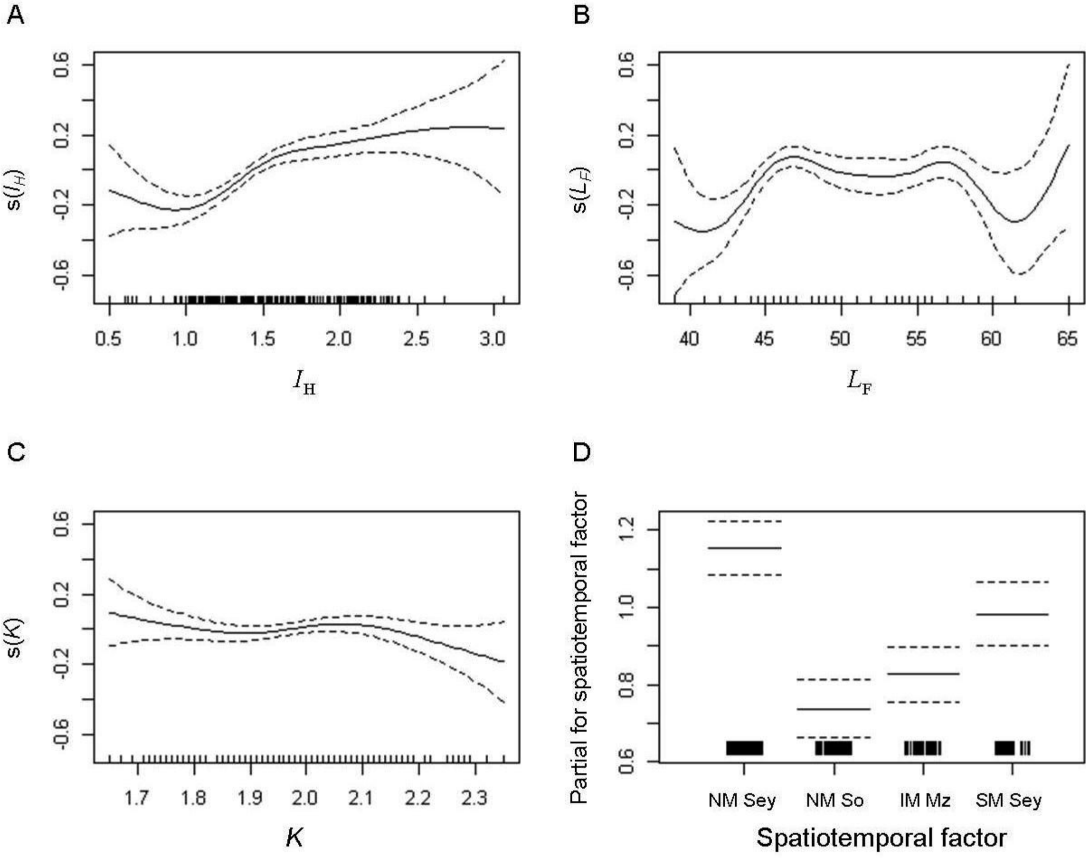

Fig. 7.6. Panel A, B and C show the estimated effect on I_G of I_H, L_F and K respectively by a solid line and 95% confident limits are shown with dashed lines. Panel D show the estimated effect for each level of the spatiotemporal factor: NM Sey, North-east monsoon in Seychelles; NM So, North-east monsoon in Somalia; IM Mz, inter-monsoon in Mozambique Channel; and SM Sey, South-west monsoon in Seychelles.

Discussion

According to the results, the sex ratio of skipjack in the Western Indian Ocean was 1:1, which was in accordance with the data obtained by Cayré and Farrugio (1986) for skipjack in the Atlantic Ocean. When the sex ratio was estimated for 5-cm size classes, it did not significantly differ from 1:1. Other studies, such as Stéquert and Ramcharrun (1996) and Timohina and Romanov (1996), showed a predominance of males in larger fish, which is a common characteristic of tropical tuna worldwide (Timohina and Romanov, 1996). Although not statistically significant, this pattern was also observed in this study. There could be various causes for the differences in number between sexes as the fish grow: differences in the mortality rate for males and females, different behaviour or being more vulnerable to fishing (Stéquert and Ramcharrun, 1996; Schaefer, 2001*b*; Lambert *et al.*, 2003).

The size (i.e., fork length) at maturity (*L*$_{50}$) of females was estimated to be 39.9 cm. Other studies in the same area (Stéquert and Ramcharrun, 1996; Timohina and Romanov, 1996) reported higher values. For example, Stéquert and Ramcharrun (1996) estimated the length at maturity (*L*$_{50}$) as 42 cm for females and 43.5 cm for males. The difference between our estimates and the ones given in other studies could be due to which stage was selected to represent mature individuals. In this work, the appearance of CA oocytes in the ovaries was considered to be an indicator of the sexual maturity (Lowerre-Barbieri *et al.*, 2011*a*). However, if we apply the classification proposed by other authors (Stéquert and Ramcharrun, 1996), which considers females to be mature when they have oocytes in the Vtg3 or GVM stage as the most advanced oocyte type in the gonad, our *L*$_{50}$ would not differ from the one previously reported (Stéquert and Ramcharrum, 1996). Nevertheless, taking into account the quick oocyte development rates of this species (Grande *et al.*, 2012), it seems more appropriate to use the CA stage as the maturation threshold (Wright, 2007; Lowerre-Barbieri *et al.,* 2011*a*). The CA stage has been associated with the timing of the physiological trigger (Wright, 2007; Brown-Peterson *et al.*, 2011; Lowerre-Barbieri *et al.,* 2011*a*). Its function is to make vitellogenesis possible, and thus in species with annual reproductive cycles and when there are no reports of high numbers of females resorbing cortical alveolar oocytes (Lowerre-Barbieri *et al.*, 2011*a*), as is the case of the skipjack*,* females with CA oocytes generally continue with the oocyte development process (i.e., vitellogenesis and maturation).

In species with indeterminate fecundity, such as skipjack (Grande *et al.*, 2012), the annual fecundity should be calculated by estimating of the number of oocytes per batch, the percentage of females spawning per day (i.e., spawning fraction), and the duration of the spawning season (Murua and Saborido-Rey, 2003). The F_{B} estimates for skipjack ranged from 100,828 to 627,325 oocytes. The mean F_{Brel} was estimated as 140 ± 64 oocytes g^{-1} of fish. Compared with previous studies in the Indian Ocean (Stéquert and Ramcharrun, 1995) and other areas (Cayré and Farrugio, 1986; Goldberg and Au, 1986), the batch fecundity values are in the data range of the previous estimates (i.e., 100,000 and 1 million eggs). F_{Brel} was close but slightly higher than that estimated by Stéquert and Ramcharrum (1995) for the Indian Ocean (i.e., 40 to 130 eggs g^{-1}, with a mean of 110eggs g^{-1}). As there were no histological indicators of imminent or recent spawning (i.e., the presence of hydrated oocytes and POF, respectively) it was not possible to assess the spawning frequency with the available methods: hydrated oocyte and POF methods (Hunter and Goldberg, 1980), visual census (Asoch, 2003), using the gonadosomatic index as a proxy (Claramunt and Herrera, 1994) and the method based on the vitellogenic oocyte growth rate (Ganias *et al.*, 2011). This is because the POFs are quickly resorbed, i.e. in less than 24h (Hunter *et al.*, 1986), at high water temperatures (Kurita *et al.*, 2011) and fishing is carried out in the morning, whereas spawning usually occurs at night (Hunter *et al.*, 1986; Schaefer, 2001*b*). Consequently, at the time of fishing the POFs are indistinguishable.

Based on the histological examinations it was observed that around 70% of the female population was in the spawning capable phase from January 2009 to May 2010. In addition, the mean I_G value by month showed that a large part of the mature female population had entered the spawning season during the sampling period. There was never more than 20% of females in the regressing phase during the sampling, and the highest numbers were recorded in February and March (i.e., 17.1% and 15.5% respectively), i.e., the NM. Therefore, like Stéquert and Ramcharrun (1995 and 1996) and Stéquert *et al.* (2001), we suggest that skipjack in the Western Indian Ocean spawns all year round. This type of long spawning season is commonly seen in warm-water habitats. The duration of the spawning season tends to become longer inversely to the latitude, with some tropical fish spawning all year round (Wright and Trippel, 2009). The seasonal variation of I_G in combination with the histological analysis showed two peaks in spawning activity: one from January to February (i.e., NM), a minor spawning period during March (i.e., NM) and from April to May (i.e., IM), and the second peak from June to July (i.e., SM). Therefore, it seems that the reproduction of skipjack in the Western Indian Ocean could be affected by climatic events, as suggested by Stéquert and Ramcharrun (1995, 1996) and Stéquert *et al.* (2001), and the peaks could be related to the NM and SM, which correspond to high productivity periods (Schott *et al.,* 2002; Wiggert *et al.,* 2006). Phytoplankton blooms have been found to be a response to the Somalia current flowing pole-ward during SM along the eastern coast of the African continent and the divergence zone in the north edge of trades during the SM and NM (Veldhuis *et al*., 1997; Schott and Mc. Creary, 2001; Schott *et al.,* 2002; Wiggert *et al.,* 2006). The zooplankton biomass therefore increases which results in large aggregations of potential prey (Roger, 1994*b*) that could enhance feeding activity and hence lead to an increase in the reproductive capacity of skipjack in response to the high metabolic rate of the species (Essington, 2003).

Demographic differences were observed in the reproductive timing of the population. These differences have been related in other species to the spawning experience, resulting in an extended spawning season for the fish in the oldest year class (Kjesbu *et al.*, 1996). Nevertheless, contrary to previous observations made for skipjack in the Indian Ocean*,* in which reproductive success is positively related to fish size (Stéquert and Ramcharrun, 1995), the seasonal variation of I_G suggest that the fish ranging from 45 to 50cm, which could be considered to be in their first spawning year, make a higher reproductive investment. This is in line with the slow growth rate observed in the fish from 45 cm onwards (Eveson *et al.*, 2012). This behaviour could be an adaptation to high mortality rates, both natural and anthropogenic due to fishing, in order to support the population by making the largest energy investment for spawning during the first spawning year. The removal of the older members of the stock could lead the younger age classes to make a bigger investment in egg production in order to compensate for the high mortality (Rochet *et al.*, 2000; Yoneda and Wright, 2004). This adaptive strategy could lead to a minor investment in reproductive output in the subsequent spawning events (Kjesbu *et al.*, 1996).

In the fish close to spawning, I_G was highly variable in the same length/gutted-weight class in accordance to previous observations in the Atlantic Ocean (Cayré and Farrugio, 1986; Goldberg and Au, 1986). In addition, fish size did not explain much of the variance in the batch fecundity, unlike the findings of Stéquert and Ramcharrum (1995), who did observe a positive relationship. In our case, batch fecundity was highest in intermediate size classes. In females close to spawning, the variations in I_G could be influenced by different factors, such as the physiological condition of the fish (Marshall *et al.*, 1998), energy consumed during spawning (Dominguez-Petit *et al.*, 2010), environmental factors (Korta *et al.*, 2010*b*), population density (Kim *et al.*, 2006) and past investments in reproduction (Kjesbu *et al.*, 1996). In the present study, the fork length, the hepatosomatic index, Fulton´s condition factor, and the spatiotemporal factor (the highest I_G values were observed during the NM and SM in the Seychelles) together explained 58.7% of deviance of the I_G. Therefore, the individual reproductive capacity depends on the season in which the fish was captured, i.e., individuals at different stages in the sequence of egg batch production (Wright and Tripple, 2009), which in turn could influence the fish condition. As the spawning season progresses the F_B (Murua and Motos, 2006) and egg size may decrease depending on the species (Kjesbu *et al.*, 1996). Therefore, the batch fecundity, and hence I_G, may vary greatly for a fish with the same length/gutted weight depending on the capture date during the spawning season, i.e., at the beginning or at the end of the individual spawning season (Korta *et al.*, 2010*b*). It does not necessary correspond to the peak and lowest point in the population's spawning activity as reflected by the high I_G observed in the IM in the Mozambique Channel. It has been observed in other fish species that larger and more viable eggs are produced earlier during the individual’s spawning season (Marteinsdottir and Begg, 2002). Therefore, as the spawning cycle progresses fish energy reserves decrease (Hunter and Leong, 1981), resulting in lower investment in reproduction and in the reallocation of energy (Stearns, 1992). This could explain the negative relationship between I_G and K. However, as mentioned, results show that proportionally more energy is allocated to reproduction in the first spawning year than in larger females, regardless of the season.

The information gained from the condition indices suggests that when skipjack enters its individual spawning season the liver plays an important role in fat storage and mobilization. The seasonal variations of the condition indices, although masked by the population's asynchronous spawning (Dominguez Petit *et al.,* 2010), and the relationship between I_G, I_H and K in fully developed fish suggest a change in energy allocation from the muscle to the liver and gonads. However, the variations in K, although statistically significant, were small. Therefore, it is probable that reproductive success is only weakly dependent on the energy stored in the muscle, contrary to the observations made in other tunas (Dotson, 1978). In addition, it is possible that the energy storing process is not a mechanism for supplying future reproductive demands, unlike the strategy adopted by cold-water species that store energy during periods of surplus resources (i.e., capital breeders) (Pedersen and Hislop, 2001). Previous studies have found that the maturation of the gonad does not affect the biting response, with the exception of fish with ripe eggs (Yuen, 1959), and that growth in large fish (i.e., somatic growth and gonadal development) is limited by their ability to consume and process food (Kitchell *et al.*, 1978). Therefore, the high metabolic and reproductive demands of this species (Kitchell *et al.*, 1978; Hunter *et al.*, 1986; Essington, 2003) in the epipelagic and oligotrophic waters of the Indian Ocean (Wiggert *et al.*, 2006) could be fulfilled by continuous feeding during the spawning season. Consequently, the gonad size could increase at the expense of the lipid supply acquired from the food intake (Johnson, 2009) (i.e., income breeders) during the individual spawning season, which may be accumulated and repackaged in the liver (Wiegand, 1996; Johnson, 2009), contrary to what is observed in capital breeders (i.e. at the expense of the energy reserves). Therefore, the seasonal variation in the reproductive activity could be related to shifts in prey and diet composition (Jaquemet *et al.,* 2011) driven by monsoon events (Schott and McCreary, 2001; Schott *et al*., 2002) or eddies in the Mozambique Channel (Tew Kai and Marsac, 2010). Anti-cyclonic eddies passing through the Mozambique Channel drive the distribution and transport of nutrients and induce upwellings supporting the food chain in the Channel (Tew Kai and Marsac, 2010). A similar relationship was suggested for yellowfin tuna feeding intensively during the spawning season in the Atlantic Ocean (Bard *et al.,* 2002).

The physiological indices analysed in this study (i.e., the I_H and K), although suitable for analysing the nutritional status of the fish (Marshall *et al*., 1999), represent the variation in the liver and body size and not exactly the energy content of the animal tissues (Schulte-Hostedde *et al*., 2005; Dominguez-Petit *et al*., 2010). Therefore, and in order to corroborate the assumptions made in this study, analysing the biochemical composition of the different tissues along with the diet composition would provide insights into energy allocation during reproduction and its implications for the stock's reproductive potential.

Conclusions

This study revealed that overall sex ratio did not differ significantly from the expected 1:1 and the size at maturity (L_{50}) for females was estimated as 39.9 cm. Skipjack spawn year round with peaks in the reproductive activity during the monsoon events (i.e. North-east monsoon and South-west monsoon). However, demographic differences were detected in the reproductive timing of the population and I_G of fully developed fish (i.e. containing oocytes in Vtg3 and/or GVM stage) indicated a large investment in gamete production in fish in their first spawning year at the beginning of the individual spawning season. The batch fecundity and relative batch fecundity (140 ± 64 eggs g^{-1} of fish) were highly variable among fishes in the same size range and did not increase with fish size.

8. Chapter III

Lipids in female skipjack tuna from the Western Indian Ocean: Influence of the reproductive cycle and the fishing environment

Introduction

Lipids are organic compounds which contain fatty acids esterified to alcohol or to amino groups (Sargent *et al.*, 2002). In living organisms they occur in all cell types contributing as principle membrane's components where they are essential in maintaining the structural integrity of cells. They also serve as highly concentrated energy storages, act as chemical messengers, allow for the absorption of fat soluble vitamins, and provide shock absorption and insulation (Sargent, 1995; Wiegand, 1996; Tocher, 2003; Tocher *et al.*, 2008). In fish, lipids present other specific and fundamental functions such as in adjusting buoyancy, as integumental waterproofing, and as essential source of metabolic energy supply for swimming, growth and reproduction (Sargent *et al.*, 2002; Tocher, 2003). As an example, the investment of metabolic energy in gonad and gamete production constitutes the major energy drain, until 60% in some species (Sargent, 1995).

Three categories of lipid sources are described for the developing oocytes: (1) exogenous lipids supply through the diet, (2) endogenous lipids mobilized from the different storage fractions, and (3) lipids synthesized *de novo* in the oocyte (Johnson, 2009). The individual contribution of any of these sources depends on the environmental characteristics and the reproductive strategy adopted by the species, (Stearns, 1989). Two strategies are distinguished: the reproduction based on the concurrent food intake independent of energy storage mechanism is referred to "income breeding"; and the one dependent of the energy stores which enables decoupling feeding and reproductive periods is defined as "capital breeding". In both cases maternal nutrition affects directly the proportion of lipids in any of the storage fractions (Lambert and Dutil, 2000), as well as the quantity and quality of lipids directed to the ova development (Wiegand, 1996). Previous studies showed how fish diet and condition can significantly affect fecundity, egg quality and larval survival (Kjesbu *et al.*, 1991, 1998; Izquierdo *et al.,* 2001; Salze *et al.*, 2005). Therefore, to understand relationships between maternal nutritional status and reproductive success is essential to improve knowledge on fish population dynamics for a sustainable management (Marshall *et al.*, 2003).

Tunas are major species of pelagic ecosystems widely distributed throughout the temperate and tropical oceans that support some of the largest and most valuable of the world's fisheries, representing 12% of global capture fisheries worth US$ 5 billion a year (Majkowski, 2007; FAO, 2010). Therefore, tunas have received considerable attention during the last decades in fishery sciences to improve stock assessment and prevent overexploitation. At individual level, their reproductive and feeding ecology have been investigated mainly to develop aquaculture as an alternative to traditional fishing (e.g., Young *et al*., 1997; Mourente *et al*., 2002; Margulies *et al*., 2007; Biswas *et al*., 2009). Furthermore, tunas became priority target species in food and nutrition research to comprehend their specific fatty acid metabolism; this is particularly true for Atlantic, Pacific and Southern bluefin tunas which present the highest levels of docosahexaenoic acid (DHA) an omega 3 fatty acid known for its effects in protecting against human heart disease, and because they are highly favoured in Japan and other developed countries ("the Sashimi mania") (e.g., Ishihara and Saito, 1996; Nakamura *et al*., 2007; Morais *et al*., 2011; Popovic *et al*., 2011). However, studies that have dealt with lipid dynamics and bioenergetics during tuna's reproductive cycle are very scarce, and only concerned the emblematic Bluefin tuna, *Thunnus thynnus* (Mourente *et al.,* 2002; Chapman *et al.,* 2011; Jusup *et al*., 2011). To our knowledge, little is known on the patterns of lipid allocation to reproduction in tropical tunas, and their implications on the reproductive capacity of tuna's population.

Distributed circumtropically, the skipjack, *Katsuwonus pelamis*, is one of the most important tuna species contributing more than one half of the global catch (ISSF, 2012). Nevertheless, skipjack life history characteristics, including a low size and age at maturity, short life and high fecundity, make it resilient and not prone to overfishing compared to the other major commercial species (e.g., bluefin and albacore tunas) (Juan-Jordá et al. 2012). In the Indian Ocean, catches of skipjack increased continuously from the mid-1970s (50,000 t) to the highest level of around 600,000 tonnes in 2006. Since then, catches have been kept around 400,000 tonnes. Hence, efforts have urgently to be made to improve the scarce scientific knowledge on the biology and ecology of this highly exploited species. While the reproductive biology of *Katsuwonus pelamis* is relatively well known (Stéquert and Ramcharrun, 1995, 1996; Schaefer, 2001; Stéquert *et al*., 2001; Grande *et al*., 2012, Grande *et al*., submitted*a*), few studies focused on the lipid composition in skipjack (Saito *et al*., 1995; Watanabe *et al*., 1995; Hiratsuka *et al.,* 2004; Hiratsuka *et al.,* 2008; Intarasirisawat *et al*., 2011), or the lipid dynamics and energy allocation associated with its reproductive cycle (Kitchell *et al.,* 1978; Essington, 2003) have been published. Therefore, the main objectives of the present work were: (1) first, to characterize the lipid composition of somatic and reproductive tissues of the Indian Ocean female skipjack tunas, (2) then, to describe changes in condition indices (condition factor K, gonado- and hepatosomatic indices, I_G and I_H, respectively) and lipid composition throughout the maturation cycle of female skipjack and assess the influence of the morphometrics (fork length, L_F) and condition indices in the later, (3) evaluate the relationship of the lipid composition and egg production indices (i.e. relative batch fecundity F_{Brel} and relative number of developing oocytes *RNDO*), (4) finally, this work aimed to investigate the differences of lipid composition in females in spawning capable phase between fish caught in different fishing environments (including different areas, seasons and fish caught by different fishing strategies: fish caught under Fish Aggregating Devices ,FADs, and free swimming schools, FSCs).

Material and Methods

Sampling strategy

Surveys were conducted in 2009-2010 on board a purse-seiner fishing in FSCs and under FADs in the Western Indian Ocean (Fig. 8.1). A total of 110 skipjack females were collected from the Seychelles surrounding waters during North-east monsoon in FADs, i.e. January and February 2009 (NM Sey), from the Somalia surrounding waters during the North-east monsoon in FADs, i.e. March 2009 (NM So), from the Mozambique Channel during the inter-monsoon in FSCs, i.e. April and May 2010 (IM Mz); and from the Seychelles surrounding waters during the South-west monsoon in FADs, i.e. June and July 2009 (SM Sey) (Table 8.1 and 8.2).

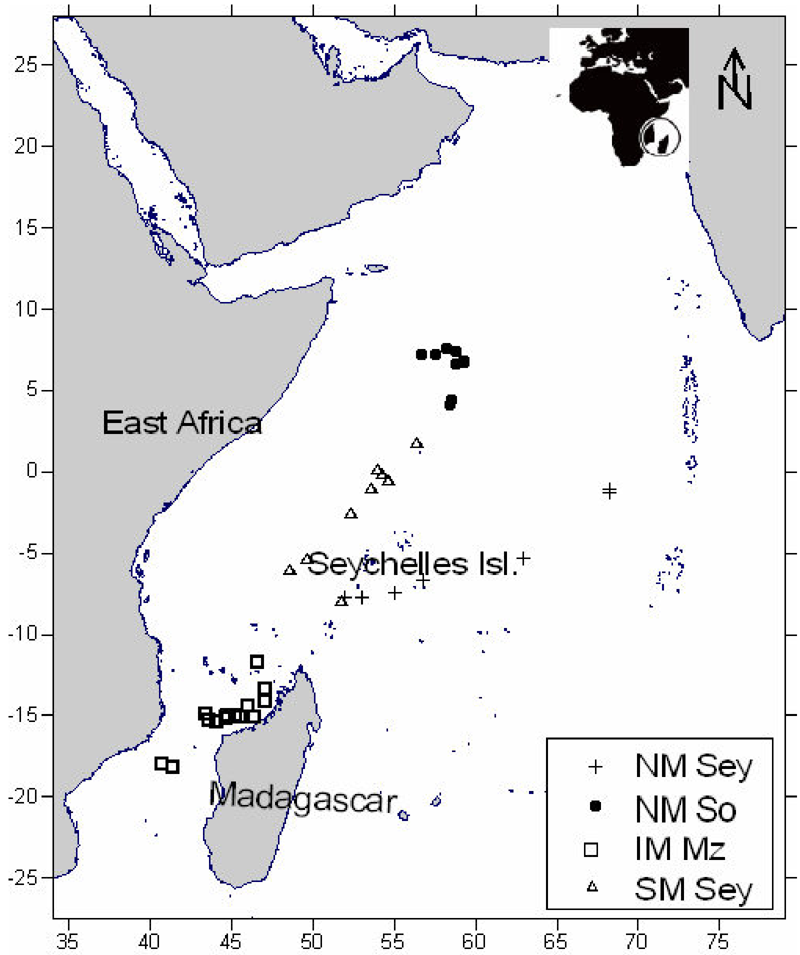

Fig. 8.1. Map of the sampling locations in the Western Indian Ocean. A total of 110 skipjack females were sampled in 2009-2010 from four areas: the Seychelles waters during the North-east monsoon under Fish Aggregating Devices (FADs), i.e. January and February 2009 (NM Sey; n=21), the Somalia waters during the North-east monsoon under FADs, i.e. March 2009 (NM So, n=24), the Mozambique Channel during the inter-monsoon in free swimming schools (FSCs), i.e. April and May, 2010 (IMS Mz; n=35), and the Seychelles waters during the South-west monsoon under FADs, i.e. June and July 2009 (SM Sey; n=30)

Each specimen was immediately measured (i.e., Fork length, L_F ±0.5 cm), weighted (i.e., Total body weight, M_T ±1 g; Gonad free weight, M_{Gfree} ±1 g) and eviscerated. The recorded masses of the liver (M_L±0.1g) and the gonads (M_G±0.1g) allowed to determine three condition indices: gonadosomatic index I_G corresponds to the ratio of gonad weight to somatic gonad free weight ($I_G=M_G\times M_{Gfree}^{-1}\times 100$); hepatosomatic index I_H corresponds to the ratio of liver weight to somatic gonad free weight ($I_H=M_L\times M_{Gfree}^{-1}\times 100$); and Fulton's condition factor K corresponds to the ratio of somatic gonad free weight over the cube of fork length ($K= M_{Gfree}\times(L_F^3)^{-1}\times 100$).

Table 8.1. Mean (±standard deviation) of morphometrics, condition indices and mean (±standard error) total lipid contents in female skipjack tunas collected from the Western Indian Ocean. Females are classified in different reproductive phases according to the most developed oocytes present in the ovary: PG = primary growth; CA = cortical alveolar; Vtg1, Vtg2, and Vtg3 = primary, secondary, and tertiary vitellogenesis, respectively; GVM = germinal vesicle migration oocytes; Reg = ≥ 50% of oocytes in atretic stage; n = number of samples; L_F = Fork length; M_T = Total body weight; I_G = Gonadosomatic index; I_H = Hepatosomatic index; K = Fulton's condition factor; $\mathrm{TL}_{\mathrm{Liver}}$, $\mathrm{TL}_{\mathrm{Muscle}}$, $\mathrm{TL}_{\mathrm{Gonads}}$ = Total lipids in liver, muscle and gonads, respectively.

Reproductive phase	**Oocyte stage**	***n***	L_F (*cm*)	M_T (*kg*)	K	I_G	I_H	**$\mathrm{TL}_{\mathrm{Liver}}$** ($\mu g.mg^{-1}ww$)	**$\mathrm{TL}_{\mathrm{Muscle}}$** ($\mu g.mg^{-1}ww$)	**$\mathrm{TL}_{\mathrm{Gonads}}$** ($\mu g.mg^{-1}ww$)
Immature	*PG*	9	43.8±7.8	1.7±1.1	1.9±0.1	0.5±0.2	1.3±0.4	8.9±0.2	4.2±0.2	5.3±0.2
Developing	*CA*	10	45.1±6.9	1.9±1.3	1.9±0.2	0.8±0.5	1.2±0.4	12.8±0.2	4.1±0.2	6.5±0.2
	Vtg1	11	47.6±7.6	2.3±1.2	1.9±0.2	1.1±0.6	1.1±0.5	13.0±0.2	5.1±0.2	9.4±0.2
Spawning capable	*Vtg2*	21	47.0±6.6	2.3±1.0	2.0±0.1	1.7±0.5	1.3±0.3	19.4±0.1	5.4±0.1	17.2±0.1
	Vtg3	22	48.4±5.7	2.4±1.0	2.0±0.2	2.3±0.8	1.5±0.4	14.8±0.1	3.6±0.1	19.5±0.1
	GVM	26	47.1±3.6	2.2±0.6	2.0±0.1	3.1±1.0	1.8±0.5	19.8±0.1	5.5±0.1	18.0±0.1
Regressing	*Reg*	11	51.2±6.4	2.9±1.1	2.0±0.1	1.4±0.5	1.1±0.2	16.3±0.2	5.6±0.2	13.9±0.2

Table 8.2. Mean (±standard deviation) of morphometrics, condition indices and total lipid (±standard error) contents determined in spawning females of skipjack tuna from the Western Indian Ocean. The fishing environment was specified, such as the fishing location and season (NM Sey = Seychelles waters during the North-east monsoon, i.e. January and February 2009; NM So = Somalia waters during the North-east monsoon, i.e. March 2009; IM Mz = Mozambique Channel during the inter-monsoon, i.e. April and May 2010; SM Sey = Seychelles waters during the South-west monsoon, i.e. June and July) and the school type (FADs = Fish Aggregating Devices; FSCs = Free Swimming Schools). *n* = number of samples; L_F = Fork length; M_T = Total body weight; I_G = Gonadosomatic index; I_H = Hepatosomatic index; *K* = Fulton's condition factor; TL_{Liver}, TL_{Muscle}, TL_{Gonads} = Total lipids in liver, muscle and gonads, respectively.

Fishing location and season	**School type**	***n***	L_F *(cm)*	M_T *(kg)*	***K***	I_G	I_H	TL_{Liver} ($\mu g.mg^{-1}ww$)	TL_{Muscle} ($\mu g.mg^{-1}ww$)	TL_{Gonads} ($\mu g.mg^{-1}ww$)
NM Sey	FAD	17	48.2±3.2	2.3±0.5	2.0±0.1	3.3±1.1	1.7±0.5	12.0±2.0	9.8±2.0	14.1±2.0
NM So	FAD	12	50.4±7.9	2.9±1.0	2.0±0.1	1.9±0.7	1.3±0.2	18.4±2.2	4.8±2.3	18.9±2.3
IM Mz	FSC	19	46.3±1.2	1.9±0.1	1.9±0.1	2.3±0.8	1.8±0.3	40.2±2.1	6.2±2.0	29.7±1.9
SM Sey	FAD	17	46.3±7.4	2.2±1.3	2.0±0.2	2.1±0.8	1.2±0.3	18.4±2.0	3.3±2.0	21.1±2.0

For histological and fecundity analyses, a sub-sample of the posterior right lobule of gonads was collected and preserved in a solution of 4% buffered formaldehyde. For lipid analysis, approximately 10g of gonads, liver and white muscle were collected and stored at -80°C for conservation until further analysis.

Histological analysis

All gonad samples were histologically processed to determine skipjack reproductive phase as described by Grande *et al.* (2012). Four reproductive phases were identified based on the presence of 7 oocyte developmental stages: immature phase containing oocytes in primary growth stage (PG); developing phase containing oocytes in cortical alveolar (CA) or primary vitellogenic stage (Vtg1); spawning capable phase in which the most developed oocytes were in vitellogenic stage (Vtg2 and Vtg3 = secondary and tertiary vitellogenesis, respectively) or in germinal vesicle migration stage (GVM). Finally, the regressing phase (Reg) corresponded to specimen with ≥ 50% of the oocytes in atretic stage.

Estimation of the reproductive capacity

Although the skipjack is considered a batch spawner species with indeterminate fecundity (Grande *et al.,* 2012), the relative number of developing oocytes (*RNDO),* defined as the number of oocytes larger than CA, was quantified to evaluate the effect of lipid composition in the reproductive capacity of skipjack females. It was determined on spawning capable females with most advanced oocytes in Vtg3 stage (n=21) by counting the follicles from CA to Vtg3 stages present in whole-mount samples (0.04±0.001 g) with image analysis (Thorsen and Kjesbu, 2001). In addition, the relative batch fecundity F_{Brel} was determined on actively spawning females in GVM stage (n=23) by the gravimetric method (Brown-Petersen *et al*., 2011; Grande *et al.,* submitted*a*).

Lipid class analysis

Each 10g-sample was first cryo-grinded as fast as possible to avoid lipid degradation by using a mixer mill MM400 Retsch® (Verder, France). A sub-sample

(0.1±0.001g) of the frozen homogenized powder was then weighted under nitrogen atmosphere, and extracted according the method of Folch *et al.* (1957). Briefly, 4mL of Folch mixture (i.e., dichloromethane:methanol, 2/1, v/v) were added, and lipids were extracted with a potter homogenizer (glass/Teflon) at 0°C. After addition of 1mL of aqueous KCl (0.88%, w/v), the extract was further vortexed, centrifuged at 2000rpm, allowing for the collection of the lower organic phase. The procedure was repeated twice to ensure a whole recovery of total lipids. Finally, the lower organic phase was evaporated to complete dryness with a RapidVap® vacuum at 35°C (Labconco, Fisher Scientific, France). The final volume of the total lipid (TL) extract then was adjusted to 2mL with dichloromethane.

Lipid classes were quantified using an Iatroscan MK-VI (Iatron laboratories) thin-layer chromatography-flame-ionization detector analyzer (TLC–FID). Aliquots of TL were spotted on quartz chromarods SIII covered with silica, and separated into triacylglycerols (TAG), free sterols (ST), sterol- and wax-esters (SE-WE), ketones (KET), acetone mobile polar lipids (AMPL) and phospholipids (PL) (Parrish 1987, 1999). Concentrations of lipid classes are expressed in $\mu g.mg^{-1}$ on a wet weight (ww) basis. Total lipid content ($\mu g.mg^{-1}$ ww) corresponds to the sum of lipid classes, and allowed to estimate the relative contribution (%) of each lipid class to the total fat.

Statistical analysis

The Kruskal Wallis test was applied to study the variability of fish condition indices (K, I_G and I_H) between reproductive phases. Mann-Whitney U test was used for comparison of significant effects.

Analyses of covariance (ANCOVAs) were applied first to compare changes in lipid composition in somatic and reproductive tissues over the reproductive cycle of female skipjack tunas (Model A), then to apprehend the effects of the fishing environment on lipids distribution in liver, gonads and muscle of spawning females (Model B). In both models, tissue was used as a random variable, and L_F, I_G, I_H and K as covariates. In model A, the categorical fixed variable corresponds to the reproductive phases defined by 7 oocyte developmental stages (PG, CA, Vtg1, Vtg2, Vtg3, GVM and Reg). In model B, it corresponds to 4 different fishing environments that take into account the fishing location and season (NM Sey, NM So, IM Mz and SM Sey), and the school type (FADs *vs* FSCs) due to the high correlation between these factors (Stéquert *et al.,* 2001; Grande *et al.,* submitted*a*). Finally, the relationships between the batch fecundity (F_{Brel}), *RNDO* and the lipid class composition in gonads, liver and muscle of female skipjack were investigated using a stepwise regression model. Residual were screened for normality by using normal probability plot and Shapiro-Wilk test. When necessary data were log+1 transformed to achieve the normality of residuals and homogeneity of variance. Analyses were carried out using SAS (SAS Institute Inc., Cary, NC, USA).

Results

Biological characteristics of female skipjack: morphometrics, gonad maturation and condition

Information on morphometrics, condition indices and tissue fat content determined in female skipjack collected from the Western Indian Ocean is summarized in Table 8.1. The sampling covered the entire reproductive cycle of skipjack, from immature (ovaries containing oocytes in PG stage) to actively spawning capable phase (ovaries containing the most developed oocytes in GVM stage) and regressing phase (indicating the end of the individual reproductive cycle). The biological characteristics of the collected fish are presented in Table 8.1. Female fork length ranged from 33 to 65 cm (L_F = 47.4±6.3 cm, n = 110). The gonadosomatic index ranged from 0.3 to 5.2 (I_G = 1.9±1.1), and varied significantly over the reproductive cycle ($H(6, 110)=71.8$, $p<0.001$) with a clear increment from minimum average value in immature (I_G = 0.5±0.2) and maximum in actively spawning capable females in GVM (I_G = 3.1±1.0) to decrease again in regressing phase (Table 8.1). The hepatosomatic index ranged from 0.3 to 3.1 (I_H = 1.4±0.5) and also showed significant differences between reproductive phases ($F(6, 110)=4.8$, $p=0.03$): I_H decrease from immature (I_H = 1.3±0.4) to reach minimum values in developing females (containing oocytes at Vtg1 stage: I_H = 1.1±0.5), then to increase until the maximum values observed in spawning capable individuals (I_H=1.8±0.5) as observed for I_G (Table 8.1). Finally, the Fulton's condition factor K fluctuated between 1.7 and 2.7 (K=2.0±0.2) without any significant variation between reproductive phases ($H(6, 110)=11.3$, $p=0.08$)

Lipids in female skipjack tunas over the reproductive cycle

Total lipids determined in female skipjack showed the highest concentrations in liver and gonads compared to white muscle (Table 8.1). In general, TAG, PL and SE-WE were predominant accounting together for 80%, 70% and 75% of TL content in gonads, liver and muscle respectively. However, TL content and lipid class distribution in somatic and reproductive tissues varied significantly over the reproductive cycle of female skipjack (Table 8.3A). Detailed concentrations of lipid classes ($\mu g.mg^{-1}$ ww) measured in the gonads, liver and white muscle of females at different reproductive phases are given in the Annexe 1. Overall, the lowest TL contents in all tissues analysed were determined in immature females that mainly store fat in liver (PG: 8.9±0.2 $\mu g.mg^{-1}$ ww) (Fig. 8.2). As such, immatures present lipid-poor ovaries (PG: 5.3±0.2 $\mu g.mg^{-1}$ ww) and an increase of TL content was observed together with maturation until a maximum measured in ovaries of spawning capable females (Vtg3: 19.5±0.1 $\mu g.mg^{-1}$ ww). A similar trend was observed in liver although significant differences were noted only between immature and spawning capable females ($F=3.24$, $p=0.001$). By contrast, white muscle in females was characterized by low and relatively constant TL levels over the reproductive cycle (average concentration of TL = 4.8±0.1 $\mu g.mg^{-1}$ ww). The significant effect of the interaction reproductive phase × tissue on TL distribution in skipjack females was also observed for PL and SE-WE. Concentrations of SE-WE in gonads increased from 0.1±0.3 $\mu g.mg^{-1}$ ww in PG stage to 5.1±0.1 $\mu g.mg^{-1}$ ww in Vtg3 stage; those of PL increased significantly from 1.8±0.1 $\mu g.mg^{-1}$ ww in Vtg1 stage to 5.6±0.1 $\mu g.mg^{-1}$ ww in Vtg3 stage. Similar trends were observed in liver for both lipid classes. As regards SE-WE, the lowest concentrations were measured in non-reproductive females (PG: 0.4±0.2 $\mu g.mg^{-1}$ ww) to reach maximum values in the liver of spawning capable individuals (Vtg2: 2.7±0.1 $\mu g.mg^{-1}$ ww; $F=3.12$, $p=0.002$). However, it is noteworthy that these maximum SE-WE levels in liver were still 2-fold lower than values measured in ovaries of spawning capable females ($F=2.42$, $p=0.01$). Female skipjack had low and similar PL levels in somatic tissues during the first stages of gonad maturation (i.e., from PG to Vtg1). While concentrations in white muscle remained below to 2 $\mu g.mg^{-1}$ ww over the reproductive cycle, PL increased in liver with the initiation of the vitellogenesis (Vtg1) to reach the highest values in spawning capable females (Vtg2: 3.5±0.1 $\mu g.mg^{-1}$ ww), followed by a decrease at Vtg3 stage

(2.3±0.1 $\mu g.mg^{-1}$ ww) and relatively constant levels until the end of skipjack reproductive cycle (Fig. 8.2).

Other lipid classes were influenced by maturity and/or tissue, without significant interaction between the two variables (Table 8.3A). Hence, immature females (PG) presented the lowest TAG concentrations, while the highest were measured in actively spawning individuals (GVM); no significant differences of TAG levels were noted for the other oocyte maturation stages (Fig. 8.2). Despite very low concentrations, AMPL and KET seemed to increase with gonad development until Vtg2, followed by highly variable levels in the advanced stages. Finally, TAG and ST presented significant differences of tissue distribution independently of the skipjack reproductive cycle, the main storage tissues being the liver and the gonads, respectively.

Relationships between condition and lipids in female skipjack were investigated (Table 8.3A). Neither the fork length (L_F) nor the Fulton's condition factor (K) was correlated with the lipid class concentrations in somatic and reproductive tissues. On the contrary, the gonadosomatic index I_G was positively correlated with TL content measured in the three tissues (Gonads: $R^2=0.51$, $p<0.001$; Liver: $R^2=0.06$, $p=0.01$; White muscle: $R^2=0.06$, $p=0.01$), as well as with PL and SE-WE concentrations in gonads ($R^2=0.41$, $p<0.001$ and $R^2=0.41$, $p<0.001$). Significant relationships were also observed between the hepatosomatic index I_H and the concentrations of TL and PL determined in liver (TL: $R^2=0.20$, $p<0.001$; PL: $R^2=0.08$, $p<0.001$), white muscle (TL: $R^2=0.07$, $p=0.007$; PL: $R^2=0.09$, $p=0.001$), and gonads (TL: $R^2=0.15$, $p<0.001$; PL: $R^2=0.12$, $p<0.001$), as well as between I_H and SE-WE levels in gonads and liver ($R^2=0.12$, $p<0.001$, and $R^2=0.18$, $p<0.001$, respectively), and KET levels in liver ($R^2=0.13$, $p<0.001$).

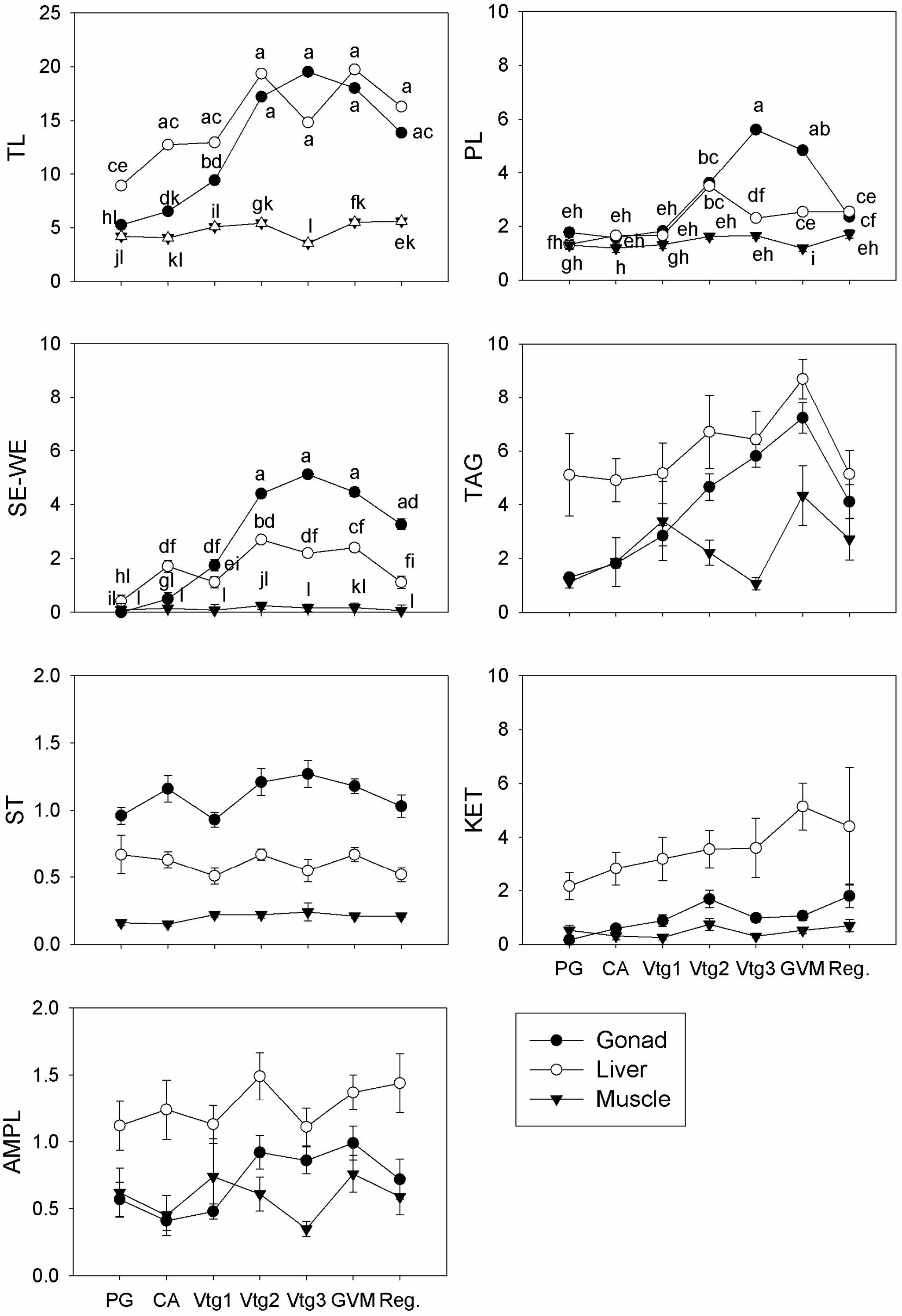

Fig. 8.2. Changes in lipid class concentrations (µg.mg^{-1} ww; mean±SE) in somatic and reproductive tissues of female skipjack tunas over the reproductive cycle. The solid circle, white circle and triangle represent fluctuations in gonads, liver and muscle, respectively. PG = primary growth; CA = cortical alveolar; Vtg1, Vtg2, and Vtg3 = primary, secondary, and tertiary vitellogenesis, respectively; GVM = germinal vesicle migration; Reg = regressing phase.

Table 8.3. Summary from the split-plot ANCOVA models using fish fork length (L_F), Fulton condition factor (K), hepato- and gonadosomatic indices (I_H and I_G) as covariates. The model (A) tested effects of reproductive phase, tissue and the interaction reproductive phase × tissue on lipid composition in female skipjack tunas; the model (B) tested effects of fishing environment, tissue and the interaction fishing environment × tissue on lipid composition in spawning capable female skipjack tunas.

(A)

Source of variation	**d.f**	**SE-WE**		**KET**		**TAG**		**ST**		**AMPL**		**PL**		**TL**	
		F	***p***	**F**	***p***	**F**	***P***	**F**	***p***	**F**	***p***	**F**	***p***	**F**	***p***
Reproductive phase	6	6.44	**<0.0001**	3.7	**0.0023**	3.4	**0.004**	1.3	0.2659	3.66	**0.0026**	3.41	**0.0041**	6.38	**<0.0001**
Tissue	2	4.37	**0.0138**	0.72	0.4882	5.15	**0.0066**	8.02	**0.0004**	2.75	0.0666	2.75	0.0664	7.68	**0.0006**
Interaction	12	3.02	**0.0007**	1.4	0.0509	1.77	0.0559	1.2	0.2861	0.8	0.652	3.53	**<0.0001**	2.72	**0.002**
L_F x Tissue	3	0.23	0.8731	0.60	0.6134	2.36	0.0725	1.40	0.2451	2.56	0.0564	0.30	0.8230	1.57	0.1986
I_G x Tissue	3	2.89	**0.0365**	1.16	0.2837	2.57	0.0551	0.62	0.6040	1.36	0.2546	6.63	**0.0003**	3.38	**0.0193**
I_H x Tissue	3	11.42	**<0.0001**	12.42	**<0.0001**	1.09	0.3530	1.06	0.3654	1.33	0.2662	8.85	**<0.0001**	8.22	**<0.0001**
K x Tissue	3	1.42	0.2370	1.39	0.2463	0.81	0.4873	2.25	0.0831	0.14	0.9344	1.92	0.1267	1.64	0.1821

(B)

Source of variation	**d.f**	**SE-WE**		**KET**		**TAG**		**ST**		**AMPL**		**PL**		**TL**	
		F	**P**	**F**	**P**	**F**	**P**	**F**	**P**	**F**	**P**	**F**	**P**	**F**	**P**
Fishing environment	3	16.38	**<0.0001**	9.38	**<0.0001**	4.82	**0.0046**	3.88	**0.0136**	2.27	0.0904	47.11	**<0.0001**	23.98	**<0.0001**
Tissue	2	3.17	**0.0459**	0.89	0.4134	2.91	0.0586	6.48	**0.0022**	1.56	0.2140	0.39	0.6785	1.51	0.2262
Interaction	6	6.98	**<0.0001**	4.15	**0.0008**	6.03	**<0.0001**	12.24	**<0.0001**	4.02	**0.0011**	2.13	**0.0456**	10.29	**<0.0001**
L_F X Tissue	3	0.54	0.6543	1.07	0.3629	0.15	0.9290	1.12	0.3442	0.10	0.9597	0.84	0.4725	0.64	0.5922
I_G X Tissue	3	2.99	**0.0338**	0.94	0.4236	7.27	**0.0002**	5.62	**0.0012**	3.76	0.0127	17.37	**<0.0001**	10.01	**<0.0001**
I_H X Tissue	3	2.09	0.1048	0.31	0.8197	3.35	0.0214	3.95	**0.0099**	1.98	0.1201	3.01	**0.0331**	2.74	**0.0466**
K X Tissue	3	1.13	0.3390	0.10	0.9578	1.10	0.3519	1.56	0.2032	0.58	0.6317	0.78	0.5071	0.55	0.6514

Significant probabilities are in bold. SE+WE, KET, TAG, AMPL and PL were log+1 transformed.

Effects of the fishing environment on lipids in spawning female skipjack tunas

The effects of the fishing environment were studied on spawning capable skipjack with ovaries in spawning capable phase containing oocytes in Vtg2, Vtg3 or GVM stage, and characterised by fork length from 33 to 63 cm (L_F = 47.6±.5.5 cm; *n*=66). As regards their condition indices, I_G (*H*(3, 66)=16.09, p=0.001) and I_H *(F*(3, 66)=11.21, p<0.001) varied significantly between fishing seasons and areas. The former presented the highest values during NM in Seychelles, while the average highest I_H was observed in fish caught in the Mozambique Channel (Table 8.2). The lowest values were found during SM in Seychelles and NM in Somalia for both I_H and I_G (Table 8.2). On the opposite, *K* did not present significant variations among fishing environments (*H*(3, 66)=6.60, p=0.08).

Detailed concentrations of lipid classes (µg.mg^{-1} ww) measured in the gonads, liver and white muscle of females caught in different fishing environments are given in the Annex 2. All lipid classes varied significantly as function of fishing environment x tissue (Table 8.3B). In general, similar values of TL, SE-WE, KET and TAG were observed in gonads of fish caught during NM and SM in Seychelles and Somalia (Fig. 8.3). Significant differences between these fishing environments were only appreciated for PL, showing the lowest values during NM in Seychelles. The highest concentrations of lipids (i.e. TL, SE-WE, KET, TAG and PL) in gonads were observed in female caught in FSCs during the IM in the Mozambique Channel. Other lipid classes such as ST and AMPL presented low concentrations and did not show substantial variations. Similar trends were observed as regards liver: levels in the same range were measured during NM and SM seasons and the highest values were observed during the IM in Mozambique Channel (Fig. 8.3). Finally, the fishing environment had no effect on the muscle lipid concentrations, except for TAG showing significant lower levels in the muscle of female skipjack caught during the NM in Seychelles.

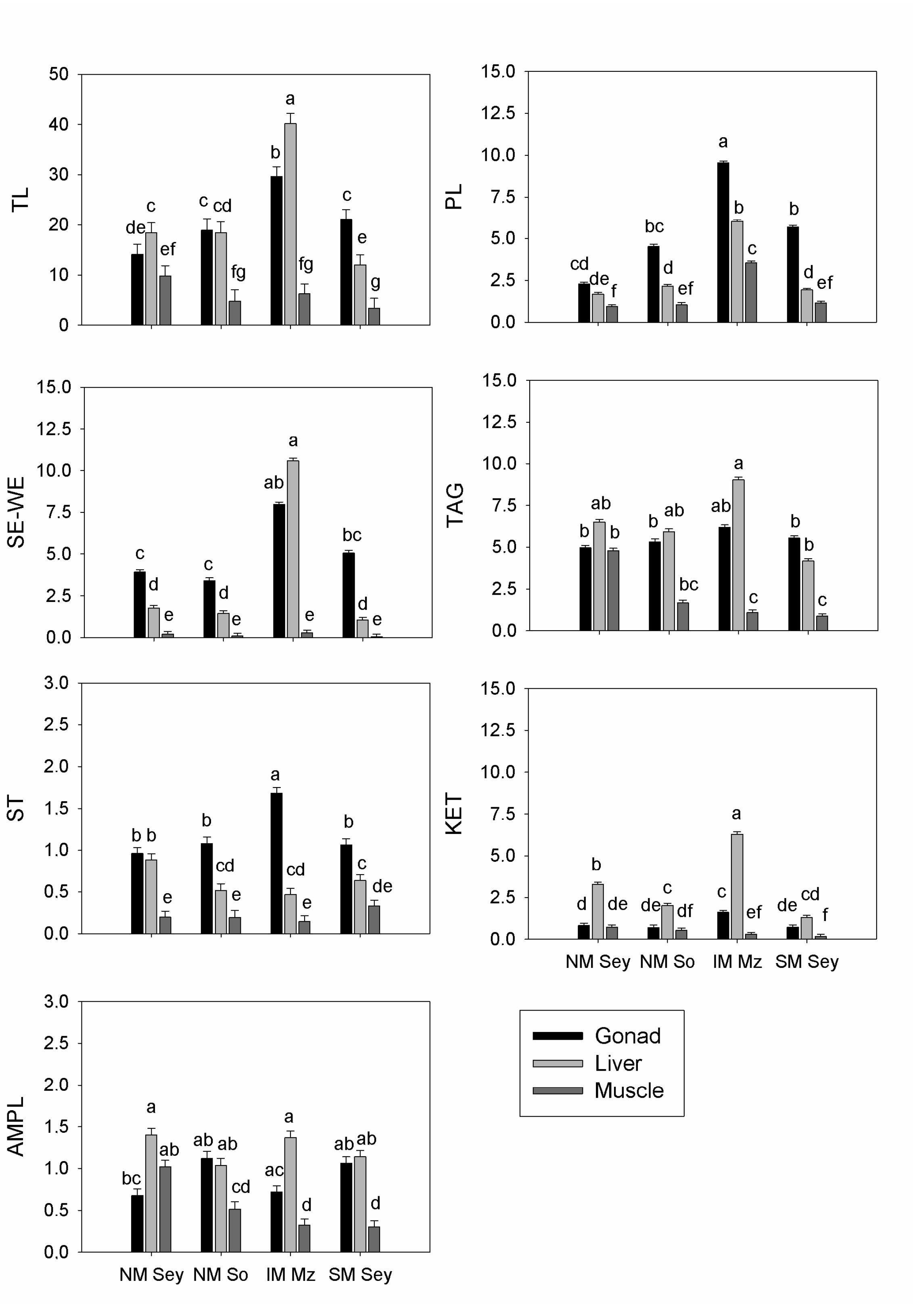

Fig 8.3. Changes in lipid class concentrations (µg.mg^{-1} ww; mean±SE) in somatic and reproductive tissues of spawning capable female skipjack tunas in relation with the fishing environment (i.e., location, monsoon season and school type). IM Mz = Mozambique Channel during the inter-monsoon; SM Sey = Seychelles waters during the South-west monsoon; NM Sey = Seychelles waters during the North-east monsoon; SM So = Somalia waters during the North-east monsoon; FADs = Fishing Aggregating Devices; FSCs = Free swimming schools.

The investigation of the relationships between fish condition and tissue lipid concentrations in spawning capable females (Table 8.3B) highlighted positive correlations between I_G and SEWE (R^2=0.08, p=0.0188), TAG (R^2=0.26, p<0.0001), PL (R^2=0.13, p=0.003) and TL (R^2=0.16, p=0.0001) measured in gonads, as well as between I_G and TAG in the liver (R^2=0.07, p=0.003). Similarly, I_H was positively correlated with TAG (R^2=0.13, p=0.003), PL (R^2=0.06, p=0.042) and TL (R^2=0.17, p=0.0008) in the liver, as well as with TAG (R^2=0.07, p=0.036) and TL (R^2=0.12, p=0.005) in the muscle. On the contrary, lipid classes in somatic and reproductive tissues were related neither with K nor with L_F.

Relationships between tissue lipid composition and skipjack reproductive capacity

Results of the stepwise regression model showed that SE-WE, ST and TAG in gonads partially explained fluctuations on F_{Brel}, resulting in an R^2 of 0.61 (Table 8.4). By contrast, only TAG in gonads was included in the analysis (F=9.0; P=0.0074) when studying *RNDO*, resulting in an R^2 of 0.32.

Table 8.4. Summary of the stepwise selection introducing (A) the relative batch fecundity (F_{Brel}) and (B) the relative number of developing oocytes (*RNDO)* as dependent variable.

(A)

Step	Variable	R^2	F	P
1	TAG	0.2255	6.11	0.022
2	ST	0.3688	4.54	0.0457
3	SE-WE	0.6069	11.51	0.0031

(B)

Step	Variable	R^2	F	P
1	TAG	0.3214	9.00	0.0074

Discussion

The skipjack tuna from the Western Indian Ocean has a protracted spawning season and asynchronous spawning behaviour at population level (Stéquert *et al.*, 2001; Grande *et al.,* submitted*a*). These reproductive characteristics can mask the temporal variations on the energy allocation and mobilization of reserves as observed in hake (Korta *et al.*, 2010*b*; Dominguez-Petit *et al.*, 2010). Therefore, in order to evaluate the energy allocation strategy adopted by this tuna species it seems more appropriate to assess the energy dynamic during the sexual maturation cycle rather than the seasonal variability (Dominguez-Petit *et al.,* 2010). Considering that lipids are the most important energy source for fish compared to proteins and carbohydrates (Sargent, 1995), the study of the lipid composition during skipjack maturation can help in understanding its energy requirements and allocation strategy. In addition, it can bring some insights regarding the importance of condition indices in this tuna species.

The total lipid content measured in reproductive tissues of skipjack showed that the lipid transfer to the ovary starts in developing females with oocytes at CA stage, surely by circulating very low density lipoproteins VLDL, and continues during the vitellogenesis phase as previously described in teleost fish (Patiño and Sullivan, 2002; Lubzens *et al.,* 2010). Lipids are accumulated in lipoproteins and oil globules in the ovary as it ripens (Wiegand, 1996). The levels of total lipids measured in skipjack gonads, from 1.0 to 5.2% of wet weight, were in accordance with those reported in scombrids (Saito *et al.,* 1997; Hiratsuka *et al.*, 2004; Intarasiriwat *et al.,* 2011) and slightly lower than those observed in other marine fish such as cod, herring and haddock (Tocher and Sargent, 1984). Thus, skipjack eggs can be considered as having low lipid content (i.e. <5% of wet weight), a common feature of marine and pelagic species (Tocher, 2003). Total lipid content in ovaries approximately followed the same trend as did the gonadosomatic index I_G with skipjack maturation. In addition, I_G was positively correlated with PL, SE-WE and TAG levels measured in gonads during maturation. Significant relationships were also observed between I_G and the concentrations of TAG, PL and TL measured in gonads of spawning capable females. Therefore, I_G is assumed to be a good indicator of the skipjack tuna reproductive capacity.

Overall, neutral lipids predominated over polar lipids in skipjack ovaries as previously observed in temperate scombrids, such as *Sarda sarda* and *Thunnus thynnus* (Mourente *et al.*, 2002; Ortega and Mourente, 2010). In particular, TAG and SE-WE are present in high concentration in eggs containing lipid droplets, with the highest levels noticed in ovaries of spawning capable female skipjack (Tocher, 2003). Both lipid classes serve as energy reserves and their amount is positively related to the elapse time between fertilization and hatching (Tocher and Sargent, 1984), with a preferential use of TAG over SE-WE (Wiegand, 1996; Tocher *et al.*, 2008). SE-WE also serve to maintain pelagic eggs' position in the water column (Johnson, 2009). In skipjack, the elapse time between fertilization and hatching (24 h) and the duration of the yolk sac larva stage (48 h) are short (Matsumoto *et al.,* 1984), responding to the low fat content in ovaries which is surely an adaptation to the high predation (Bailey and Houde, 1989). In addition, previous studies have demonstrated egg positive buoyancy until few hours before hatching in *Katsuwonus pelamis, Thunnus albacores* and *Euthynnus linneatus* (Matsumoto *et al.*, 1984; Margulies *et al.*, 2007). In skipjack ovaries, PL quantity also increases from the initiation of vitellogenesis. PL are structural lipids essential as membrane components and allow to maintain the viscosity and cellular enzymatic activity. However, they can also serve as a source of energy in developing embryo (Tocher *et al.,* 2008). Although PL, TAG and SE-WE are considered as the main structural and/or major energetic reserves for fish embryo and larvae development (Sargent, 1995), other lipids may contribute as energy source in gamete production such as sterols and ketones. ST may also contribute to cell structure and function and is indispensable for steroid synthesis (Perevozchikov, 2008). Concentrations in skipjack ovaries were low and relatively stable over reproductive cycle, but were relate with egg production indices. As regards KET, the highest levels observed in females at the second vitellogenic stage and those in regressing phase may indicate periods of major energy expenditure or exhaustion since these marine lipids are known to mainly occur in fasting conditions (Tocher, 2003). The present lipid data measured in skipjack reproductive tissues allowed describing the general picture of energy allocation to gonad maturation, but it does not allow to investigate the lipid dynamic or to identify the sources of each lipid class to oocytes. According to the literature, neutral lipids (e.g. TAG and SE-WE) in the oocytes are more influenced by the diet than polar lipids (e.g. PL) suggesting an exogenous source supplied from fish diet (Watanabe *et al.*, 1995;

Johnson, 2009); but endogenous sources from reserves (Wiegand, 1996; Tocher, 2003) and/or *de novo* synthesis in the liver and oocytes (Seo, 2001; Johnson, 2009; Lubzens *et al.*, 2010) were also reported in teleosts. PL are biosynthesized in fish liver or mobilized from other tissues to be deposited in the liver, and then transferred to oocytes as lipoproteins, e.g. vitellogenin (Sargent, 1995; Wiegand, 1996; Tocher *et al.*, 2008; Johnson, 2009).

The lipid composition in skipjack liver was influenced by the reproductive cycle. In particular, phospholipid and neutral lipid (TAG and SE-WE) concentrations showed important fluctuations over maturation which were likely to respond to reproductive demands. Such results seem to be reinforced with the positive relationships obtained between the hepatosomatic index and the total lipid contents measured in liver and gonads. The liver plays an important role in clearing the fatty acids from the plasma and repackaging the ones derived from food or other storage tissues prior to the transfer to the oocyte (Wiegand, 1996; Johnson, 2009). Indeed, the lipoproteins biosynthesis takes place, leading to a relative size increase of the liver (Sargent, 1995) as reflected by the I_H *vs* liver TL correlation. Although organ's relative mass variability not necessary reflect fluctuations of fat reserves over the reproductive cycle (Schulte-Hostedde *et al.*, 2005), the correlation between I_H and gonad TL levels put in light that this physiological condition index could represent the synthesis or accumulation of lipids and lipoprotein assembly which may be closely related to reproduction requirements (Wiegand, 1996). After spawning, the partial weight of the liver was reduced indicating the organ regression which may be related to spawning cessation.

White muscle of skipjack tuna was characterized by low lipid concentrations (< 5% of wet weight) compared to other tissues as shown previously (Medina *et al.*, 1995; Saito *et al.,* 1997). skipjack can thus be defined as "lean fish" with a preferential storage of fat reserves in liver. Furthermore, the absence of significant fluctuations of lipid concentrations in skipjack muscle over the reproductive cycle suggested the weak role of this organ as lipid supplier for gonad maturation. As highly migratory species, tunas present a specific high aerobic capacity in white muscle allowing for burst swimming (Korsmeyer and Dewar, 2001). Mourente *et al.* (2002) suggested that white muscle lipid depot is more likely devoted to sustain bluefin tuna swimming activity rather than to fulfil its energetic requirements for reproduction.

The energy storage occurs as an anticipate respond to predictable changes that could affect fish survival or reproductive success (Bonnet *et al.,* 1998). In temperate fish facing low productivity periods, the storage of high amounts of fat prior the reproduction period is a common characteristic (Sargent, 1995). In tropical and subtropical waters, seasonal changes in temperature, photoperiod and food resources are less abrupt (Bye, 1984) and fish may not have to anticipate to low rations. According to the observed variability of lipid composition over its maturation cycle and its reproductive traits (Grande *et al.,* 2012; submitted*a*), the skipjack tuna seems to be oriented towards an "income" spawning strategy. Indeed, no major lipid accumulation occurred in the muscle and liver before the onset of the spawning, and in the same way no lipid depletion was observed during the ovarian growth. Therefore, the ovary ripening may occur mainly at the expense of the food intake, as shown in species with indeterminate fecundity and asynchronous oocyte development, such as hake and gilthead seabream (Almansa *et al*., 2001; Dominguez-Petit *et al*., 2010). The serial spawners continue feeding through the spawning and incorporate more dietary lipids in maturing oocytes (Jonhson, 2009). Vitellogenesis is thus mainly carried out at the expense of the food intake and not only at the expense of energetic reserves (Dominguez-Petit *et al.,* 2010). The absence of lipid depletion in the principal energy depots during maturation can be attributed to a lack of endogenous lipid supply from liver and muscle to the maturing ovary as observed in white seabream (Perez *et al.,* 2007). The indeterminate fecundity type adopted by the skipjack (Grande *et al*., 2012) leads to a continuous recruitment of oocytes during extent spawning periods, which can last for 3 months (Schaefer, 2001). The yolk material is continuously deposited in the ovaries as long as the fish remains in the spawning capable phase. It involves a continuous lipid supply from the liver, which may increase the repackaging or *de novo* biosynthetic activity to fulfil the increasing requirements of lipids in the ovaries (Tocher, 2003; Johnson, 2009). Therefore, it could act as a buffer concurrently gaining resources from the diet (Bonnet *et al*., 1998). In this way, skipjack may increase its productivity in periods of high food availability (Bye, 1984; Margulies *et al.,* 2007; Bapary *et al.,* 2012). Experiments in captive yellowfin (*Thunnus albacares*) strengthened such assumption with an increase in egg production observed in response to increasing daily rations (Margulies *et al.,* 2007).

Fluctuations in dietary lipids due for example to changes in prey availability or environmental conditions could negatively impact maternal energetic status and fecundity (Kjesbu *et al.,* 1991; Kjesbu, *et al.,* 1998), egg quality (Salze *et al.,* 2005), and therefore could affect offspring development and survival (Izquierdo *et al.,* 2001). The present study highlighted the influence of the fishing environment on the liver and gonad lipid composition of spawning capable skipjack females. In particular, the highest lipid contents were found for females caught in free-swimming schools during the inter-monsoon season (from April to May 2010) in the Mozambique Channel, while similar concentrations were measured in females caught under FADs in the other fishing areas (the Seychelles and Somalia surrounding waters during the North-east and South-west monsoon seasons). As demonstrated in previous works focused on the feeding ecology of tropical tunas, the occurrence of empty stomach in fish caught around drifting FADs is a common fact, especially for skipjack tuna (Ménard *et al*., 2000; Potier *et al.,* 2001). Such a result was attributed to tunas being trapped by FADs which have a detrimental effect on fish diet (Jaquemet *et al.,* 2011) and condition (Hallier and Gaertner, 2008). However, the present results do not allow demonstrating a clear effect of FADs on the lipid composition and thus energetic status of reproductive female skipjack due to an unsuitable temporal and spatial stratification of the sampling. First, the monsoon circulation occurring in the Western Indian Ocean and the particular circulatory characteristics of the Mozambique Channel result in seasonal and spatial differences in primary productivity (Schott *et al.,* 2002; Wiggert *et al.,* 2006), and consequently, in differences of abundance of tunas' common preys between the fishing areas such as *Cubiceps pauciradiatus* (Potier *et al*., 2008), *Natosquilla investigatoris* (Jaquemet *et al.,* 2011) and *Charybdis smithii* (Romanov *et al.,* 2009). Indeed, the variability in lipid composition observed in female skipjack could simply reflect differences in prey availability and thus dietary lipids among the fishing areas and seasons. Furthermore, since the early 1990s, tropical tuna purse-seine fisheries have widely enhanced the use of FADs in the Indian Ocean to increase their catch (Dagorn *et al.,* 2012, Fonteneau *et al*., 2013). The oceanic circulation and the strong seasonal pattern of FAD deployment (i.e., mean number of FADs deployed per vessel 2.5-fold higher during the North-east monsoon in the Somalia surrounding waters; Fonteneau *et al.,* 2013) have resulted in a modification of tuna's habitat with waters north 7ºS characterized by a higher density of FADs. Although the results here seems in accordance with previous works suggesting a negative effect of FADs on tuna's condition, trophic ecology, and reproduction (Hallier and Gaertner, 2008; Marsac *et al.,* 2000; Ménard *et al.,* 2000; Jaquemet *et al.,* 2011; Dagorn *et al*., 2012; Fonteneau *et al.,* 2013), further investigations have to be urgently conducted to specifically test the hypothesis that FADs may act as "ecological traps" for tunas. In particular, a huge effort will have to be made on the tuna sampling design in order to be separate the fishing mode effect from the fishing area and season effects.

As regard tuna's reproductive capacity, both estimators used in this work (i.e. F_{Brel} and *RNDO*) were positively related to neutral lipid levels in the gonads, while no significant relationships were observed with liver and muscle fat content. In relation to the income breeding strategy, we wonder that the positive correlation observed between I_H and gonad lipid levels in fish in spawning capable phase, rather than showing the relationship between the reproductive capacity and hepatic stores, was a response of the transitory accumulation of lipids in the liver and high vitellogenic activity of the tissue during the spawning capable phase (Bonnet *et al*., 1998; Rinchard and Kestemont; 2003). As mentioned, dietary lipids are exported *via* lymphatic and circulatory systems or *via* the portal system to the liver (i.e. exogenous transport), and then are transported as lipoproteins to storage and utilization tissues (i.e. endogenous transport) (Sheridan, 1988; Tocher, 2003). As such, liver is generally described as a short term storage tissue working as an interface between the exogenous and endogenous transport (Sheridan, 1988; Tocher, 2003), and being strongly affected by prey lipid composition oppositely to muscle and gonads (Wiegand, 1996; Tocher, 2003; Saito *et al*., 2005). Fecundity is not always equally sensitive to food deprivation or increase (Wootton, 1998), and fatty acid composition of eggs is more resistant to dietary changes than other tissues (Tocher, 2003). Margulies *et al.* (2007) highlighted an increase of *Thunnus albacares* egg production on a short time period (<4-21 days) in response to daily ration increases. The reproductive strategies of both tropical tuna species are similar (Grande *et al.,* 2012; Zudaire *et al.,* 2013) and, thus this result bring some interrogations about the skipjack and the sensitivity of this species to food deprivation. In addition, female nutrition may also affect the egg quality and not uniquely the fecundity (Izquierdo *et al.,* 2001). Hence, the influence of skipjack trophic ecology on its reproductive behaviour and fecundity still need to be further investigated, especially through cultured tank experiments in order to better understand and predict the effects of global changes on tuna populations.

Conclusions

According to the variations observed in the lipid composition of somatic and reproductive tissues during the maturation cycle, skipjack tuna can be defined as “lean fish” and seems to lie towards the income breeding strategy in the continuum of capital/income. On the one hand, gonad and liver TL, TAG, SE-WE and PL levels significantly increase wih ova maturation, and the highest TL contents of hepatic and reproductive tissues were measured in fish in spawning capable phase. In the same, time muscle was characterized by approximately 3-fold lower levels than other tissues and did not reflect significant variations during the reproductive cycle. Therefore, the reproduction seems to be financed with the energy gained concurrently. On the other hand, as common fact among income breeders *RNDO* and F_{Brel} were related to lipids in the gonads, unlike somatic tissues. Finally, changes in the lipid composition were observed among fish caught in different fishing environment. However due to an unsuitable stratification of the sampling we could not distinguish the cause of the changes.

9. Chapter IV

Link between the reproductive activity, feeding strategy and isotopic composition of the skipjack, Katsuwonus pelamis, in the Western Indian Ocean

Introduction

Reproductive timing in fishes is regulated by endogenous factors (i.e. incompletely understood neuroendocrine mechanism, pituitary gonadotropins, sex steroids and insulin-like growth factor I) which respond to environmental cues such as temperature, photoperiod, lunar phase, rainfall, salinity, food availability and social-interactions (Brooks *et al*., 1997; Bromage *et al*., 2001; Pankhurst and Porter, 2003; Lubzen *et al*., 2010). In temperate and high latitudes the environmental factors controlling the reproduction have been largely studied (Brooks *et al*., 1997; Bromage *et al*., 2001), while little attention has been paid on fish species inhabiting tropical waters (Pankhurst and Porter, 2003). In these regions where seasonal changes in photoperiod are negligible, variations in reproductive activity of fishes are mainly driven by temperature and food resources (Bye, 1984). In the Western Indian Ocean, the monsoon seasonal wind system dominates the ocean climate and is responsible for ocean temperature and productivity changes (Schott *et al*., 2002). The South-west monsoon season (SM) is characterized by upwellings that occur north of the equator as a result of Southern Gyre and Great Whril (i.e cyclonic gyre in the 5º-12º N latitude band), and by weaker upwellings in the Arabian Sea. During North-east monsoon (NM) wind reverse and existing upwellings associated to South equatorial countercurrent (SECC). Another important area for upwelling formation during monsoons is the South Equatorial divergence zone (5º-10ºS) (Schott *et al.,* 2002).

The skipjack tuna, is oriented towards an income breeder strategy for which both ovarian ripening and spawning mainly occur at the expense of energy from food intake rather than from stored reserves (Grande *et al*., submitted*b*). Therefore, timing of maturation and breeding for this species depends on periods of high food availability such as highly productive periods during NM and SM seasons in the Western Indian Ocean. A failure in feeding success or changes in food web structure can negatively impact energy supply needed for reproduction, and consequently fish reproductive success as well as egg quality and larvae survival. Such strings of events have been observed both in experiments (Brooks *et al*., 1997; Salze *et al.,* 2005) and field studies such as for the Northern cod stocks (Lambert *et al*., 2000; Sherwood *et al*., 2007). To investigate relationships between feeding patterns and reproduction in fish is thus essential to improve knowledge on factors that affect stock reproductive potential (Trippel, 1999), and ultimately fish population resilience to fishing and other pressures such as climate change or coastal development.

Stomach content analysis (SCA) is the traditional and most convenient way of studying foraging behaviour and trophic relationships of marine species (Polunin and Pinnegar, 2002). It provides valuable and precise information about the recent diet of the animals in terms of the amount and type of food. However, due the 'snapshot' nature of sampling, the presence of unrecognized dietary items and bias from regurgitation during capture, it is now commonly recommended to combine SCA with complementary and powerful trophic tracers such as stable isotope analysis (SIA) (Polunin and Pinnegar, 2002; Reñones *et al.* 2002). Dietary studies involving stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) rely on the fact that an organism's tissue is synthesized from assimilated organic matter and reflects the isotopic signatures of the consumed prey (Fry, 2006). The consumer's tissue will be a time-integrated dietary representation on a scale of weeks to months depending on the targeted tissue (e.g., Buchheister and Latour, 2010). While δ^{13}C allows to distinguish between sources of organic matter at the bottom of food webs, $\delta^{15}N$ is closely related to animal's trophic position with a stepwise enrichment of ~3-4‰ with each trophic levels (Minagawa and Wada, 1984; Peterson and Fry, 1987). SIA has been widely used for characterizing marine food webs (Bode *et al.*, 2007), studying ontogenic diet shifts (Graham *et al.*, 2007) and describing animal movements between isotopically distinct areas (Hobson, 1999; Carlisle *et al.*, 2012). However, isotopic fractionation might also reflect metabolic processes not necessarily related to the diet, making the interpretation of isotopic data difficult (Martinez del Rio *et al.*, 2009). Animal growth (Perga and Gerdeaux, 2005), tissue elemental turnover rate (Tieszen *et al.*, 1983), and changes in food web isotopic baseline (Ménard *et al.*, 2007) are examples of the factors involved. The metabolic fluxes linked to gonad maturation might also induce changes in bulk tissue isotopic composition (Lorrain *et al.*, 2002; Schmidt *et al.*, 2004). Hence, to investigate the variability of isotopic signatures over the reproductive cycle should bring valuable insights on the energy allocation strategy adopted by the species for reproduction (Jardine *et al.*, 2005).

Despite the huge importance of the Indian Ocean skipjack fishery (ISSF, 2012; Arrizabalaga *et al.*, 2012), only few works have dealt with its feeding patterns (Roger, 1994*a,*1994*b*; Potier *et al.*, 2001; Potier *et al.,* 2002; Jaquemet *et al.,* 2011) and mainly through diet composition analysis (SCA). The present work aimed, for the first time, to investigate the possible influence of the reproductive cycle on the feeding behaviour of skipjack females by combining SCA and SIA data.

Material and Methods

Sampling

Surveys were conducted on board a purse-seiner in 2009-2010 in the Western Indian Ocean (Fig. 9.1). A total of 494 skipjack females were caught under Fish Aggregating Devices (FADs) from the Seychelles surrounding waters during North-east monsoon (i.e. January and February 2009; NM Sey), from the Somalia surrounding waters during the North-east monsoon (i.e., March 2009; NM So), from the Mozambique Channel during the inter-monsoon (i.e., April and May 2010; IM Mz), and from the Seychelles surrounding waters during the South-west monsoon (i.e., June and July 2009; SM Sey).

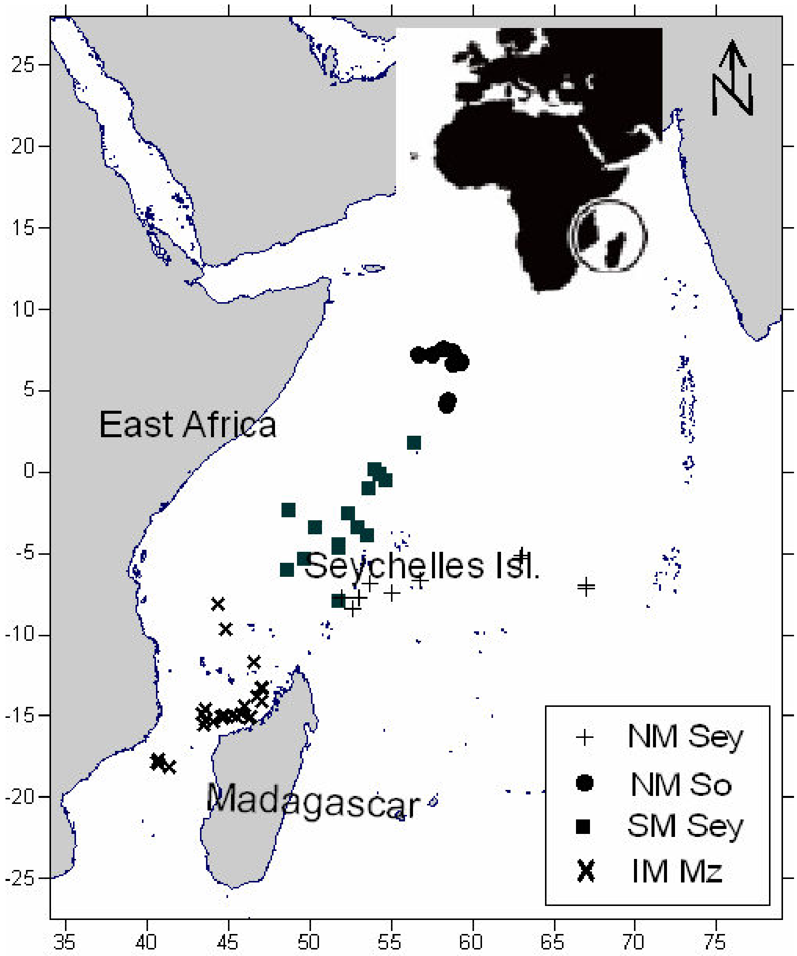

Fig. 9.1. Map of the sampling locations in the Western Indian Ocean. A total of 494 skipjack females were sampled in 2009-2010 from four areas: the Seychelles waters during the North-east monsoon, i.e. January and February 2009, and the Somalia waters during the North-east monsoon, i.e. March 2009, the Mozambique Channel during the inter-monsoon, i.e. April and May, 2010, and the Seychelles waters during the South-west monsoon, i.e. June and July 2009.

Each specimen was immediately measured (i.e., Fork length, L_F ±0.5 cm), weighted (i.e., Total body weight, M_T ±1 g) (Table 9.1), and the stomachs (n=365) were removed, labelled in plastic bags and stored at -20°C. For some specimens with empty stomachs (n=59), the anterior part of the intestine (5 cm) was preserved. In addition, samples of approximately 10 g on a wet weight basis were collected on the liver and dorsal white muscle (i.e., between the head and the first dorsal fin) of 103 skipjack females in order to conduct isotopic analysis. The weight of the gonads (M_G ± 0.01 g) was recorded for each female (n=494) allowing for the determination of the gonadosomatic index, I_G (i.e, ratio of gonad weight to somatic gonad free weight, $M_{Gfree}= M_T - M_G$) (Table 9.1), and a sub-sample of the posterior right lobule was collected and preserved in a solution of 4% buffered formaldehyde for histological analysis.

Table 9.1. Characteristics of the female skipjack collected in 2009-2010 from the Western Indian Ocean. n = number of fish; L_F = fork length (Mean±SD, cm); M_T = total body weight (Mean±SD, kg); I_G = gonadosomatic index (Mean±SD); n_S, n_L and n_M = number of stomachs, livers and muscles samples, respectively; NM Sey = Seychelles waters during the North-east monsoon; NM So = Somalia waters during the North-east monsoon; IM Mz = Mozambique Channel during the inter-monsoon; SM Sey = Seychelles waters during the South-west monsoon season.

Spatiotemporal strata	n	L_F	M_T	I_G	n_S	n_L	n_M
NM Sey	102	47.0±3.3	2.1±0.5	2.8±1.0	72	20	20
NM So	183	52.8±5.2	3.2±0.9	1.7±0.6	145	20	20
IM Mz	92	47.6±5.6	2.2±1.1	1.4±1.0	70	32	32
SM Sey	117	47.7±6.6	2.4±1.2	1.9±0.9	78	30	28

Histological analysis

Cross sections of ovaries (5 mm) were dehydrated by moving them through increasing concentrations of alcohol (i.e. from 70% to 96%) and soak in ascending solutions of resin. Then, the samples were polymerised into resin blocks. The histological sections of 3-5 µm were cut with a microtome and stained with the Harri´s Hematoxylin and Eosin. By using the microscopic slides, the ovaries were classified in different reproductive phases according to the most advanced oocyte stage present in the gonad (West, 1990) and following the classification criteria proposed by Brown-Petersen *et al.* (2011) and already applied for the skipjack (Grande *et al.*, 2012).

Stomach content analysis

At laboratory, stomachs were thawed and weighted (±0.01 g). The content was removed and sorted by broad zoological group (i.e. four prey classes: cephalopods, fish, crustacea, others). Stomachs containing only parasites were considered empty. Each prey item was counted, weighted on a wet weight basis (±0.01 g) and identified to the lowest possible taxon using identification keys (Smith and Heemstra, 1986; Clarke, 1986; Smale et al., 1995), as well as by comparison with reference specimens and/or through the identification and measurements of hard parts (e.g. otoliths, dentaries, and caudal fin for fish; beaks for cephalopods; cephalothorax, pteropods, telsons and chelipeds for crustacea). The accumulated items (i.e. eroded otoliths and cephalopod beaks without flesh) were excluded from the analysis to avoid the overestimation of some preys. Therefore, reconstituted weight (*RW*) was calculated on fresh items only. Published allometric equations between hard parts and body weight were used to estimate *RW* (Clarke, 1986; Smale *et al*., 1995; Potier *et al*., 2011). Last, the presence or absence of accumulated prey items in the intestines was also noted.

In order to investigate skipjack diet composition, three diet indices were estimated (Potier et al., 2007): (i) the frequency of occurrence (*FO*) corresponds to the number of occurrence of i prey class or species divided by the number of non-empty stomach; (ii) the mean proportion of preys by number (*%MN*) corresponds to the numerical importance of prey i calculated as number of prey class or species i divided by the total number of preys in the individual stomach and computing the mean proportions found in all stomach; finally (iii) the mean proportion of preys by reconstituted weight (*%MRW*) estimated as the reconstituted weight of prey class or species i divided by the total reconstituted weight in the individual stomach and computing the mean proportions found in all stomach. Hence, the individual fish was considered as a sampling unit. To estimate the spatiotemporal variability of stomach contents' trophic diversity (by prey classes), *N*i and *W*i were computed with the data pooled across all the stomachs. As such, mean number (*MN*) and mean reconstituted weight (*MRW*) of prey classes were computed by spatiotemporal strata. The index of stomach fullness (*ISF*) was computed as *ISF* = *M*cs/(*M*t-*M*cs) * 100, with *M*cs corresponding to the stomach content weight.

Stable isotope analysis

Each 10g-sample was first cryo-grinded by using a mixer mill MM400 Retsch® (Verder, France). A sub-sample (around 2±0.1g) of the frozen homogenized powder was then freeze-dried and the water content was determined (%). Lipids were removed by using dichloromethane on an accelerated solvent extraction system (ASE®, Dionex) as described by Bodin et al. (2009). 500±50 µg of lipid-free samples were weighted and encapsulated in a fold of tin before isotopic analysis. The ratios of carbon and nitrogen stable isotopes ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) were determined by continuous flow isotope ratio mass spectrometer (CF-IRMS). Samples were analysed using a Europa Scientific ANCA-NT 20–20 Stable Isotope Analyser, together with an ANCA-NT Solid/Liquid Preparation Module (2004–2005) and a Finnigan Mat Delta XP isotope ratio mass spectrometer interfaced with a Carlo Erba NC2500 elemental analyser (2007–2008).

As the samples contained more than 10% nitrogen, the CF-IRMS was operated in dual isotope mode, allowing $\delta^{13}C$ and $\delta^{15}N$ to be measured in the same sample. Isotopic ratios were reported in conventional delta (δ) notation as parts per mil (‰) relative to the international standards: atmospheric nitrogen (N_2) and Peedee Belemnite for $\delta^{15}N$ and for $\delta^{13}C$, respectively. Analytical precision (standard deviation; n=5) was 0.2‰ for both nitrogen and carbon, as estimated from standards (leucine) analysed together with the samples.

Statistical analysis

Differences in *MN*, *MRW*, $\delta^{13}C$ and $\delta^{15}N$ composition by spatiotemporal strata and *MN* and *MRW* by fishing time were investigated using non parametric Kruskal Wallis Tests. Mann-Whitney U test was applied to check the significant effects. The same tests were applied to evaluate the influence of the reproductive cycle on tissue isotopic composition. Note that this analysis was conducted on fish caught in SM Sey to avoid the spatiotemporal effect. Parametric tests, i.e. One-Way ANOVA and Turkey HSD test, were applied when exploring the differences in the isotopic signature among tissues by spatiotemporal strata ($\Delta\delta^{15}N_{M-L}$ and $\Delta\delta^{13}C_{M-L}$). Spearman correlation test was applied to test the relation between size of predators and preys (i.e. fish, crustacea, cephalopod and others), and tissue stable isotopic composition. Finally, the cumulative curve was computed (family level) for each spatiotemporal strata. The analysis was carried out in R software by using the “vegan” package. Rarefaction curve is read as moving from right to left and reflects the prey richness at family level for a determined sampling effort (Gotelli and Colwell, 2001).

Results

Stomach content indices in female skipjack from the Western Indian Ocean

A total of 365 stomachs were analyzed from skipjack females ranging from 33 to 68 cm (L_F=49.9±5.9 cm), from which 282 were empty (i.e., 77%). Most of the empty stomachs were encountered from 6h to 15h with a decrease of the relative number in late afternoon (Table 9.2). Generally, empty stomachs presented a contracted wall musculature suggesting a long elapsed time from the last meal. A significant influence of the fishing time on *MN* (H=80.7, p<0.001) and *MRW* (H=91.8, p<0.001) was observed, with higher values observed in the afternoon (Table 9.2). A similar result was obtained regarding the stomach fullness (H=40.2, p<0.001), with values increasing from 16h to 19h (ISF=0.6±0.7 and 1.1±1.2, respectively). Whatever the spatiotemporal stratum, ISF was not correlated with skipjack size (NM Sey, p=0.243; NM So, p=0.770; IM Mz, p=0.478, SM Sey, p=0.266). Finally, it is important to note that 78% of the analysed intestines were containing accumulated prey items.

Table 9.2. Number of stomachs (total, empty and non-empty) analysed in female skipjack caught under FADs in the Western Indian Ocean according to fishing time. Mean (±SD) of the three diet indices (frequency of empty stomachs; mean number *MN* and mean reconstituted weight *MRW* respectively) was also reported.

Local Time (h)	**Number of stomachs**			**Frequency of empty stomachs**	***MN***	***MRW***
	Total	**Empty**	**Non-empty**			
6-7	55	47	8	0.85	0.2±0.5	0.2±1.4
8-9	111	96	15	0.86	0.4±1.5	0.02±0.12
10-11	52	45	7	0.87	0.4±1.2	0.1±0.51
12-13	66	49	17	0.74	5.9±20.3	0.8±3.1
14-15	41	34	7	0.83	0.7±2.3	0.1±0.7
16-17	21	8	13	0.38	2.4±3.9	7.8±13.5
18-19	19	3	16	0.16	9.4±7.6	18.1±22.3
Total / Mean	365	282	83	0.77	2.0±9.3	1.6±7.4

Diet composition and prey size in female skipjack

24 families and 723 prey items were identified in the skipjack stomach contents, with the predominance of crustacea (Table 9.3).

Crustacea preys: Crab larvae (megalop stage) were the dominant crustacea prey accounting for 20% by number and 18% by reconstituted weight. Stomatopoda larvae (*Lysiosquilla tredecimdentata*, *Odontodactylus scyllarus* and undetermined stomatopoda larvae) were also found in appreciable numbers *(%MN*=7.4%, %MRW=7.5%). Others crustacea such as shrimps, mysidae and the swimming crab *Charibdys smithii* were observed occasionally.

Fish preys: The cigarfish *Cubiceps pauciradiatus* was the most abundant prey fish species (%*MN*=12.7%; %*MRW*=12.5%), followed by the holocentrid *Sargocentron* sp. (%*MN*=2.4%; %*MRW*=3.3%) and Exocoetidae (%*MN*=1.2%; %*MRW*=2.9%). Mean number and mean reconstituted weight of undetermined fish were not negligible (3.3% and 2.6%, respectively).

Cephalopod preys: The contribution of cephalopods to the overall diet was low. From the 19 prey items found in skipjack stomachs, *Ornithotheuthis volatilis* was the dominant species (%*MN*=1.2%; %*MRW*=1.2%).

Others preys: The pteropod *Diacavolinia* sp. was the most important species (%*MN*=22.6%; %MRW=22.2%). Other molluscs such as *Cavolinidae sp*. (%*MN*=3.4%; %*MRW*=3.1%) and *Atlanta sp*. (%*MN*=3.6%; %*MRW*=2.7%) were found in few stomachs.

The size of the prey items ingested by skipjack was in general small (Table 9.4), and was not related with skipjack fork length (crustacea, p=0.486; fish, p=0.706; cephalopods, p=0.491; others, p=0.168).

Table 9.3. Diet composition of skipjack caught around drifting FADs. Frequency of occurrence (*FO*), mean proportion by number (%*MN*) and mean proportion by reconstituted weight (%*MRW*) of prey items.

Class	**Family**	**Species**	***FO* (*n*)**	***FO* (%)**	**%*MN***	**%*MRW***
Fish			**25**	**30.1**	**22.5±39.0**	**26.4±42.9**
	Holocentridae	*Sargocentron sp.*	5	6	2.4±10.3	3.3±13.9
	Bramidae	*Brama brama*	2	2.4	0.9±7.4	0.9±6.3
	Balistidae	*Canthidermis maculata*	1	1.2	0.1±0.5	0.0±0.2
	Nomeidae	*Cubiceps pauciradiatus*	15	18	12.7±29.4	12.5±29.4
	Carangidae	*Decapterus sp.*	1	1.2	1.2±11.0	1.2±10.9
		Unknown species *(juveniles)*	2	2.4	0.6±4.1	0.9±5.9
	Myctophidae	*Diaphus sp.*	2	2.4	0.6±5.5	0.6±5.0
	Exocoetidae	*Exocoetus volitans*	1	1.2	0.3±2.7	0.5±4.3
		Unknown species	3	3.6	0.6±3.9	1.5±10.4
	Scombridae	Unknown species	2	2.4	0.6±4.1	2.2±13.9
	Mullidae	Unknown species	1	1.2	0.1±0.6	0.2±1.8
	Unknown		6	7.2	3.3±14.4	2.6±13.7
Crustacea			**42**	**50.6**	**38.2±44.6**	**34.7±44.9**
	Portunidae	*Charybdis smithii*	2	2.4	2.4±15.4	2.4±15.4
	Crab larvae	Unknown species	22	26.5	20.1±38.1	18±37.3
	Stomatopoda larvae	*Lysiosquilla tredecimdentata*	3	3.6	1.7±11.3	1.5±11.2
		Odontodactylus scyllarus	4	4.8	2.0±11.8	1.27±10.9
		Unknown species	6	7.2	1.3±6.7	1.57±10.0
	Phrosinidae	*Phrosina semilunata*	2	2.4	2.2±14.4	2.3±14.5
	Bracyscelidae	*Brachyscelus sp.*	2	2.4	0.3±2.1	0.1±1.2
	Platyscelidae	*Platyscelus ovoides*	1	1.2	1.2±11.0	1.2±10.9
	Mysidae	Unknown species	3	3.6	3.0±16.3	3.5±18.0
	Caridea	Unknown species	5	6	3.9±17.0	2.8±15.0
Cephalopods			**10**	**12.1**	**8.1±25.1**	**8.7±27.1**
	Ommastrephidae	*Sthenoteuthis oualaniensis*	2	2.4	1.3±11.0	1.3± 11.0
		Ornithoteuthis volatilis	6	7.2	3.2±14.5	3.9±18.3
	Chiroteuthidae	*Chiroteuthis sp.*	1	1.2	0.1±0.7	0±0.1
	Onychoteuthidae	*Onykia loennbergii*	1	1.2	0.1±0.7	0.5±4.2
	Unknown juveniles		2	2.4	1.8±12.2	1.91±12.6
	Unknown		1	1.2	1.2±11.0	1.2±10.9
Others			**29**	**34.9**	**31.3±44.9**	**30.1±45.1**
	Atlantidae	*Atlanta sp.*	5	6	3.6±16.6	2.7±15.5
	Cavoliniidae	*Cavolinia sp.*	4	4.8	3.4±16.6	3.1±16.2
		Diacavolinia sp.	21	25.3	22.7± 40.7	22.2±40.8
	Salpidae	Unknown species	1	1.2	0.6±5.5	1.0±9.3
	Others		1	1.2	0.4±3.7	1.1±9.7

Table 9.4. Size (mean±SD; min-max values) of skipjack prey items regrouped per class. For crustacea and other prey species: TL = Total length (mm); for fish: SL = Standard length (mm); for cephalopods: DML = Dorsal mantle length (mm).

Class	**Crustacea**	**Fish**	**Cephalopod**	**Others**
Prey size (mm)	9.4±8.7 (3-36)	44.3±29.6 (9.5-200)	50.0±14.3 (23.6-78.4)	5.5±1.3 (2.2-8.4)

Spatiotemporal variability in female skipjack diet: influence on the reproductive activity

The rarefaction curves (Fig. 9.2) were calculated (family level) for the four spatiotemporal strata and the highest richness was observed in the NM Sey (i.e. January and February). SM Sey and IM Mz presented very close richness and the lowest values were observed in the NM So.

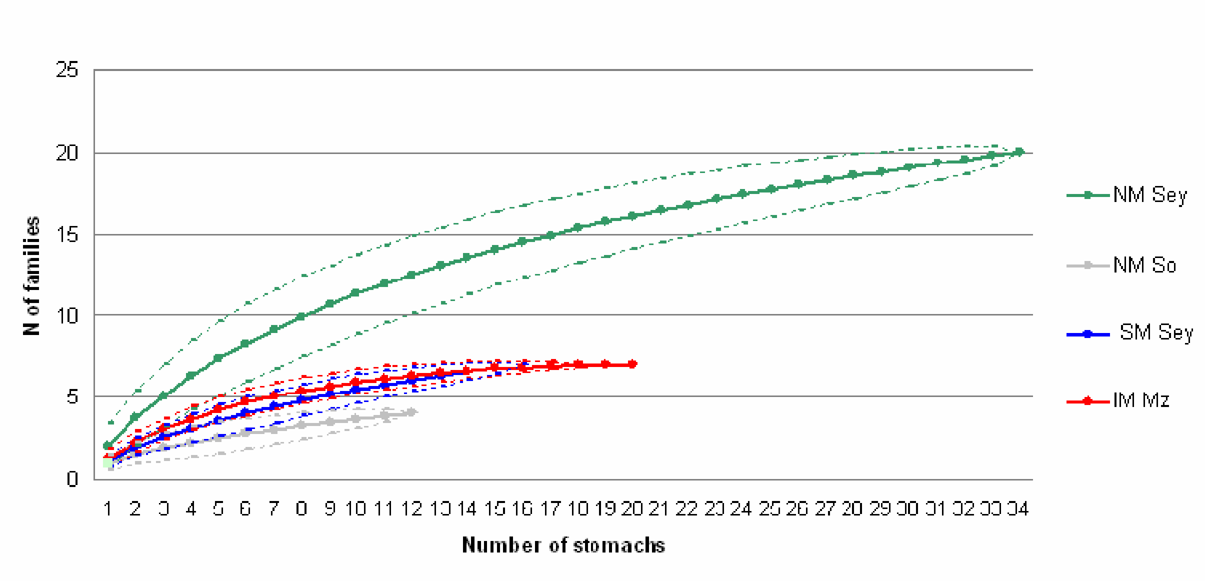

Fig 9.2. Cumulative curves calculated by spatiotemporal strata: NM Sey (January and February, 2009), NM So (March, 2009), IM Mz (April and May, 2010) and SM Sey (June and July, 2009). The rarefaction curve is read from right to left for a same sampling effort. Dashed lines indicate the confident interval at 95%.

As regards the spatiotemporal variability of the diet trophic diversity in female skipjack, significant differences of *MN* and *MRW* were noted for crustacea (H=8.9, p=0.03; H=11.1, p=0.012), fish (H=27.56, p<0.001; H=28.42, p<0.001), and others preys (H=27.9, p<0.001; H=29.2 p<0.001). The figure 9.3 represents the differences of *RW* per prey classes according to the spatiotemporal strata: Fish preys largely dominated skipjack diet (90.81%, 13.9±19.5 g) of skipjack caught in the NM Sey. Cephalopods (5.6%, 0.85±3.46 g) and crustacea (3.1%, 0.5±0.7 g) appeared in minor amounts. At this time we observed the highest I_G values (2.8±1.0). In the NM So the I_G decreased sharply (1.7±0.6). During this period others (group mainly composed by *Diacavilinia sp.*) dominated the diet by reconstituted weight (55.4%, 0.05±0.08 g). Cephalopods (29.3%, 0.03±0.09 g) and crustacea (11.3%, 0.01±0.03 g) were second and third in importance. In the IM Mz the I_G was similar (1.4±1.0) than in the NM So, but we observed an increase in the standard deviation which reflects an increase in the asynchrony in the spawning activity of analyzed fish. Crustacea contribute to 49.8% of the diet (1.1±4.7 g), cephalopods account for 31.3% (0.7±2.1 g) and 7.8% (0.4±0.9 g) the fish prey. Others were found in minor amounts 0.8% (0.1±0.2 g). Finally in fish caught in SM Sey I_G increased slightly (1.9±0.9). During the season, crustacea dominated the diet (97.3%, 1.7±2.8 g).

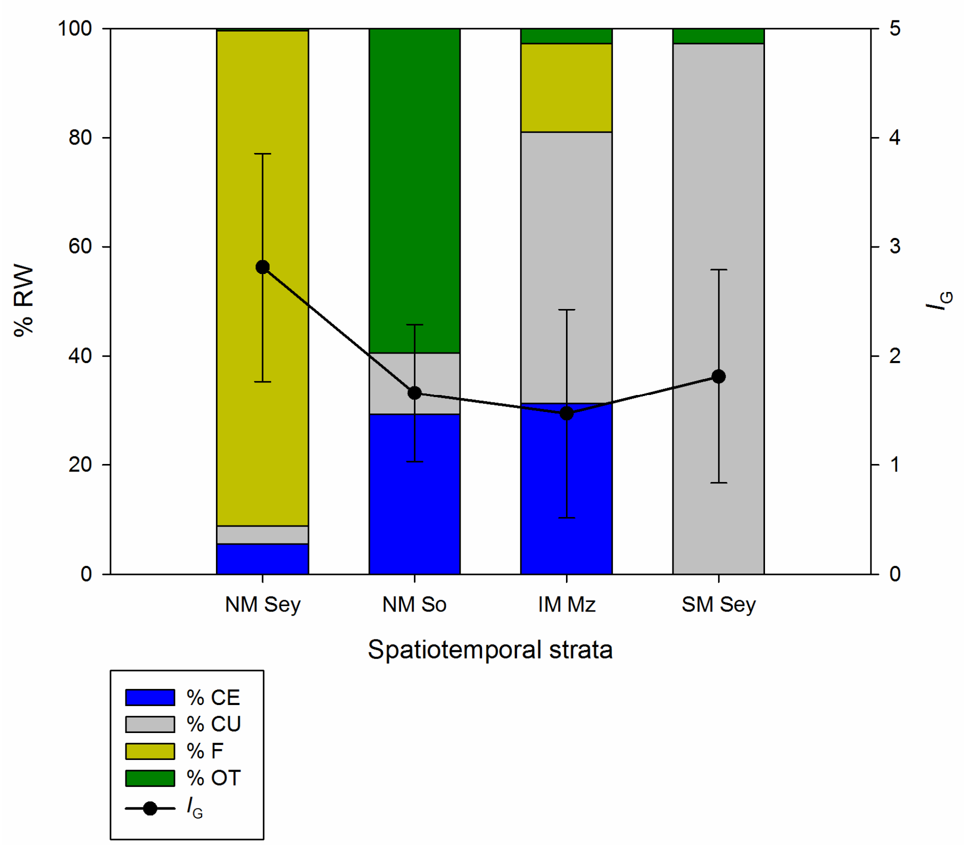

Fig. 9.3. Proportion by reconstituted weight of the four prey classes (i.e. crustacea CU, fish F, cephalopods CE and others OT) identified in the non-empty stomachs of female skipjack caught under FADs from different spatiotemporal strata in the Western Indian Ocean. The mean (±SD) of the gonado-somatic indices (I_G) calculated by spatiotemporal strata were also represented. NM Sey = Seychelles waters during the North-east monsoon (n(RW)=34, n(I_G)=102); NM So = Somalia waters during the North-east monsoon (n(RW)=12, n(I_G)=183); IM Mz = Mozambique Channel during the inter-monsoon (n(RW)=22, n(I_G)=92); SM Sey = Seychelles waters during the South-west monsoon (n(RW)=15, n(I_G)=119);

Spatiotemporal variability in female skipjack stable isotopic composition

SIA was conducted on the white muscle and liver of 103 skipjack females ranging from 33 to 63 cm (L_F=47.2±6.2 cm) (Table 9.5). $\delta^{15}N$ fluctuated from 6.4 to 12.6‰ (10.1±1.9‰) and from 8.1 to 13.2‰ (10.8±1.3‰) in skipjack liver and muscle, respectively, and $\delta^{13}C$ ranged from -18.3 to -16.8‰ (-17.6±0.3‰) and from -17.5 to -

16.1% (-16.8±0.2‰). Whatever the spatiotemporal strata, the size of the female skipjack (i.e. L_F) was not significantly correlated with $\delta^{15}N$ and $\delta^{13}C$ (data not shown).

Regarding $\delta^{15}N$, liver and muscle isotopic signatures were significantly different between the spatiotemporal strata (H=87.6 p<0.001; H=75.9, p<0.001, respectively; Table 9.5). In both tissues, the highest values were observed in the Somalia area with a decreasing gradient of 4.8‰ in liver and 3‰ in muscle southwards towards the Mozambique Channel (Table. 9.5). On the contrary, the calculated differences of $\delta^{15}N$ between tissues ($\Delta\delta^{15}N_{M-L}$) were also significantly influenced by the spatiotemporal strata (F=32.78, p<0.001; Table 9.5), with the lowest values in the Somalia area during the North-east monsoon ($\Delta\delta^{15}N_{M-L}$ = 0.41±0.49‰) and the highest in the Mozambique Channel during the inter-monsoon ($\Delta\delta^{15}N_{M-L}$ = 2.02±0.47‰) (Post hoc Tukey HSD; p=0.044 and p<0.001). In Seychelles area, skipjack presented similar $\Delta\delta^{15}N_{M-L}$ values during the North-east and South-west monsoon seasons (Post hoc Tukey HSD; p=0.972)

Carbon stable isotopes presented similar signatures in skipjack liver between the spatiotemporal strata (H=1.35; p=0.71), while weak but significant differences of $\delta^{13}C$ were noticed in the muscle (H=16.22; p=0.001) with the highest values in NM So and SM Sey and the lowest in IM Mz (Mann-Whitney U test: p<0.001 and p=0.004, respectively) (Table 9.5). Similar results were obtained for $\Delta\delta^{13}C_{M-L}$ (F=1.88; p=0.13) in the four spatiotemporal strata.

Table 9.5. Mean values (±SD) of $\delta^{13}C$ and $\delta^{15}N$ (‰) and $C{:}N$ ratio measured in the liver and white muscle of skipjack females from four spatiotemporal strata in the Western Indian Ocean. The calculated differences of carbon and nitrogen isotopic values between the muscle and the liver ($\Delta\delta^{13}C_{M-L}$ *and* $\Delta\delta^{15}N_{M-L}$, respectively) were reported. NM So = Somalia waters during the North-east monsoon, NM Sey = Seychelles waters during the North-east monsoon; SM Sey = Seychelles waters during the South-east monsoon; IM Mz = Mozambique Channel during the inter-monsoon

		NM So	**NM Sey**	**SM Sey**	**IM Mz**
Liver	*n*	20	20	30	32
	$\delta^{13}C$ (‰)	-17.5±0.33	-17.6±0.27	-17.7±0.28	-17.6±0.32
	$\delta^{15}N$ (‰)	12.02±0.55	10.7±0.42	10.0±0.48	7.23±0.5
	C:N	3.74±0.26	3.70±0.30	3.48±0.17	4.08±0.41
Muscle	*n*	20	20	28	32
	$\delta^{13}C$ (‰)	-16.7±0.17	-16.8±0.23	-16.7±0.19	-16.9±0.11
	$\delta^{15}N$ (‰)	12.3±0.79	11.6±0.41	10.9±0.55	9.32±0.61
	C:N	3.06±0.04	3.08±0.09	3.03±0.04	3.09±0.04
$\Delta\delta^{13}C_{M-L}$ (‰)		-0.86±0.25	-0.70±0.28	-0.87±0.23	-0.74±0.35
$\Delta\delta^{15}N_{M-L}$ (‰)		0.41±0.49	0.97±0.65	0.89±0.77	2.02±0.47

Changes in stable isotope composition during gonad maturation:

SIA was conducted for females caught in SM Sey to avoid the spatiotemporal effect. $\delta^{15}N$ measured in the liver and muscle of female skipjack were significantly affected by gonad development (H=9.07, p=0.03 and H=9.92, p=0.02, respectively). Liver $\delta^{15}N$ values decreased from immature (10.6±0.2‰) to spawning capable phase (9.9±0.4‰) then increased during the regressing phase (10.2±0.5‰) (Fig. 9.4). Immature fish presented higher $\delta^{15}N$ values than developing and spawning capable specimens (Mann-Whitney U test; p=0.014 and p=0.009, respectively).

An opposite trend was observed in skipjack muscle (Fig. 9.4) with significantly higher $\delta^{15}N$ values in spawning capable fish (11.2±0.6‰) than the ones in immature (10.4±0.2‰) and developing phase (10.6±0.1‰) (10.8±0.6‰) (Mann-Whitney U test; p=0.014 and p=0.012 respectively).

Finally, gonadal development did not affect $\delta^{13}C$ measured in the liver and muscle of female skipjack (H=1.02, p=0.7 and H=2.6, p=0.45, respectively) (Fig. 9.4).

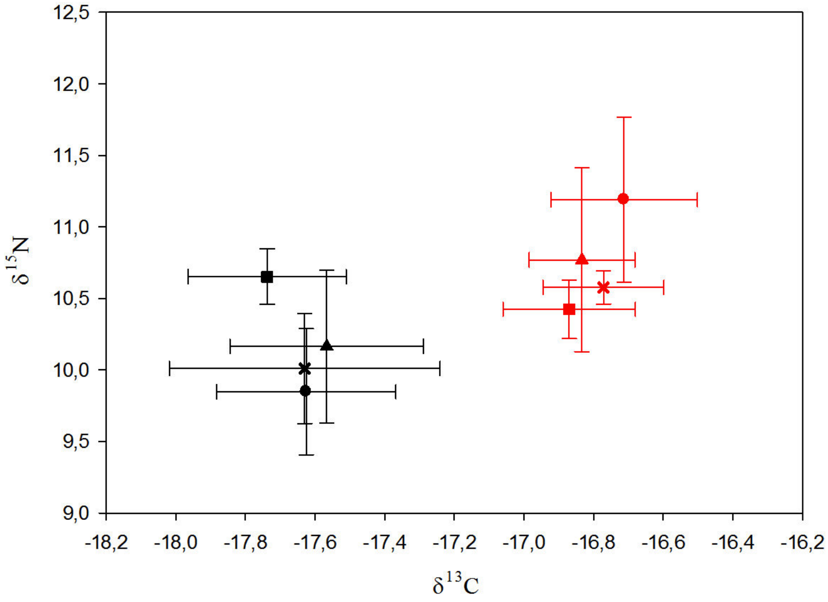

Fig. 9.4. $\delta^{15}N$ and $\delta^{13}C$ mean values (±SD) in the liver (black symbols) and the white muscle (red symbols) of female skipjack caught during the South-west monsoon in Seychelles (SM Sey) according to their reproductive phase: immature (square), developing (cross), spawning capable (circle) and regressing phase (triangle).

Discussion

Skipjack feeding behaviour under FADs in the Western Indian Ocean

In the vicinity of drifting FADs, skipjack and small yellowfin exploit the shallower layers of the water column for feeding (Potier *et al.*, 2007; Jaquemet *et al*, 2011). Dissolved oxygen and temperature limits the vertical movements of the skipjack to a more reduced space in the water column than yellowfin (Korsmeyer and Dewar, 2001; Graham and Dickson, 2004). Indeed, when assemblage of prey availability is limited, skipjack and small yellowfin could compete for the same food resource resulting in more efficient forage activity of yellowfin tuna (Jaquemet *et al.*, 2011). This inter-specific competition and low prey availability could explain the high number of empty stomach in skipjack caught around drifting FADs (Roger, 1994*a*; Ménard *et al;* 2000; Potier *et al*, 2001,2002; Jaquemet *et al.*, 2011), which could reduced the nutritional status of specimens (Hallier and Gaertner, 2008; Jaquemet *et al.*, 2011). However, if the motivation for the association is other than the trophic function, i.e. meeting point hypothesis (Robert*, et al.*, 2010), this behaviour might not have a negative effect on the biology of the population as predicted by the "ecological trap" hypothesis (Marsac *et al.*, 2000).

Roger (1994*a*) observed a day-time feeding behaviour of the skipjack around drifting FADs, while more recently night time foraging was also reported (Romanov *et al.*, 2009; Schaefer and Fuller, 2005). Also, an increase of bird foraging activity at late afternoon has been observed, which usually rely on surface predators (Weimerskirch *et al.*, 2004). Considering the fast gastric and intestine evacuation rate in skipjack (i.e., 12 h and 14 h respectively in fish fed specimens; Magnuson, 1969), the sampling strategy constrained by purse-seine fishing operations around FADs (i.e. mainly in the morning) could introduce bias in tuna trophic studies using SCA. In the present work, the number of empty stomach was the lowest at late afternoon and 78% of intestines contained accumulated prey items suggesting recent skipjack feeding. In addition, small *Charybdis smithii* (21.5±2.6 mm) in advanced stage of digestion were recovered in stomachs collected in the early morning supporting the hypothesis of a night foraging (Romanov *et al.*, 2009). Large concentrations of this prey species were found in the surface layer during night time but were never recorded down drifting FADs during the day (Romanov *et al.*, 2009). Therefore, night-time sampling effort has to be considered in the future in order to better evaluate the drifting FAD effects on skipjack foraging pattern.

Influence of skipjack diet on the spawning activity

The opportunistic feeding behaviour of tunas makes them efficient biological samplers of the micronekton (Ménard *et al.*, 2006; Potier *et al.,* 2007), and prey diversity in stomach contents could be driven by seasonal and local prey availability. Therefore, in spite of the low number of non-empty stomachs (33%), the present work gives an overall description of the seasonal variability of the skipjack diet.

The skipjack tuna is an income breeder, meaning that the energy required for gametogenesis is mainly dependent on the energy supplied directly from the diet rather than from the energy reserves (Grande *et al.*, Submitted*b*). The presence of abundant food in particular areas and/or seasons available for this species may therefore contribute to the reproductive timing (Bye *et al.*, 1984; Bapary *et al.*, 2012). Preys do not provide the same energy density to the predator (Olson and Boggs, 1986; Lawson *et* *al.*, 1998), and rich diets are important for fuelling the energetic costs of fish reproduction (Brooks *et al.,* 1997; Izquierdo *et al.*, 2001).

Previous studies described the cigarfish as the dominant prey of large tunas caught in free-schools in the Western Indian Ocean (Bashmakov, 1991; Potier *et al.,* 2008). The cigarfish is also a common prey for skipjack (Potier *et al.*, 2008). This species was observed in high densities in the eastern part of the Seychelles islands from November to January (Potier *et al.*, 2008), corresponding to the NM Sey stratum of the present study. With the predominance of north-easterly winds, upwellings are generated associated to the South Equatorial Countercurrent (SECC) and the South Equatorial Divergence leading to phytoplankton blooms (Schott *et al.,* 2002; Potier *et al.*, 2008) and, subsequently, an increase of zooplankton production (Roger *et a*l., 1994*b*). Skipjack can thus take advantage of the short food web commonly associated with large aggregations of prey fish (e.g., mainly cigarfish, as well as flying fish and holocentridae) foraging on the zooplankton (Roger *et al.*, 1994*b*). Such an abundant and high-energetic food source in NM Sey may exert a strong effect on skipjack gonad maturation, and could explain the highest I_G values and the peak of the reproductive capacity in this stratum observed by Grande et al. (submitted*a*). Previous studies already recorded an intense forage activity on cigarfish of spawning yellowfin tunas (Bard *et al.,* 2002; Fonteneau *et al.*, 2008), a tuna species exhibiting a similar reproductive strategy as *Katsuwonus pelamis* (Grande *et al.,* 2012; Grande *et al.*, submitted*a*; Zudaire *et al.,* 2013).

During the second peak of skipjack spawning activity (SM Sey) characterized by lower I_G and slightly lower reproductive capacity (Stéquert *et al.*, 2001; Grande *et al.,* submitted*a*), the number of prey items in stomachs was low and consisted of low-energetic prey such as crustacea larvae (i.e., stomatopods and megalops). These differences of diet composition in the Seychelles area between the North (i.e., mainly fish) and South (i.e., mainly crustacea) monsoon seasons might result in different investments to reproduction. Kornilova (1981) and Potier *et al.* (2004, 2007) in yellowfin, as well as Romanov and Zamarov (2007) in longnose (*Alepisaurus ferox*) noted a strong occurrence of crustacea larvae in the stomach contents related to the higher water productivity generated by the seasonal upwellings (Romanov and Zamorov, 2007). Kornilova (1981) identified the South-western part of the equatorial zone as the best feeding area for yellowfin and bigeye tunas during the South-west monsoon. Therefore, reproduction timing in skipjack might coincide with upwellings associated with either monsoon events (e.g. north-eastward upwellings at the off-shore flowing flank of the Southern Gyre, 3-5ºN; and the Great Whirl open ocean upwelling in the Arabian Sea, 5-12ºN) or the divergence zone between 5-10ºS, as previously observed in tropical species (Bye, 1984; Bapary *et al.,* 2012). On the contrary, the higher proportion of females in regressing phase in the Somalia area (NM So) suggesting a cessation of the skipjack reproductive activity in this strata (Grande *et al.,* 2012) might be linked to a weak amount of the available energy as suggesting by (i) the lower prey abundance in stomach contents, (ii) the predominance of poor-energy items (*Diacavolinia* sp), and (iii) the absence of *N. investigatoris,* a species previously reported in high abundance in tuna stomach contents in Somalian waters (Potier et al., 2002, 2007; Jaquemet *et al*., 2011).

Finally, the lower reproductive activity of skipjack females during the inter-monsoon in the Mozambique Channel (IM Mz) was in turn related to high asynchrony in the spawning activity (i.e. differences in the individual spawning season; Grande *et al.*, submitted*b*). In this region, the mesoscale rotating eddies (de Ruitjer *et al*., 2002) contribute to locally enhance the primary production (Garcon *et al.,* 2001; Vaillancourt *et al.,* 2003), impacting the biomass and the distribution of intermediate (Sabarros *et al.,* 2009) and higher trophic levels (Weimerkirch *et al.,* 2004; Tew-Kai and Marsac, 2010). During such events, the feeding opportunities of top predators increase (Weimerkirch *et al.,* 2004), and they benefit from high energy content food sources (cigarfish and cephalopods, i.e. *Ornithoteuthis volatilis* and *Sthenotheutis oualaniensis)* which could contribute to increase the reproductive capacity of fish in spawning capable phase (Brooks *et al.,* 1997; Izquierdo *et al.,* 2001). Skipjack is characterised by a high metabolism (Essington, 2003) and the energy invested in oocyte recruitment into maturing pool may be low as denoted by the early batch formation (i.e. tertiary vitellogenic stage) and the low fat content of small mature oocytes (Grande *et al.,* 2012; Grande *et al.,* Submitted*b*). Such characteristics may enable skipjack tuna to adopt a fast reproductive cycle (Hunter *et al.,* 1986; Tyler and Sumpset, 1996). Therefore, as usually occurring in fish showing indeterminate fecundity (Pavlov *et al.,* 2009; Grande *et al.,* 2012), food abundance may trigger and enhance the oocyte development in skipjack: as soon as the physiological and environmental conditions are favourable, the spawning capable ovaries begin to produce viable eggs and maintain a high rate of spawning until the environmental conditions reverse. In low latitudes, this reproductive strategy enables the individuals to spawn all year around when resources are patchily distributed (Pavlov *et al.,* 2009)

Spatiotemporal variability in female skipjack stable isotopic composition

The isotopic incorporation rate of an organism is tissue dependent and is determined by the protein turn-over rate (i.e. synthesis and catabolism) (Tieszen *et al.,* 1983; Bouchheister and Latour, 2010), the protein intake in terms of quantity and quality (Martinez del Rio *et al.,* 2009), the isotopic signatures at the base of the food web (Ménard *et al.*, 2007; Popp *et al.,* 2007), and fasting (Hobson *et al.,* 1993). In the present work, a consistent decrease in $\delta^{15}N$ signatures was observed both in liver (4.8‰) and muscle (3.2‰) of female skipjack from Somalia to Mozambique Channel. By contrast, $\delta^{13}C$ in muscle only vary 0.2% equivalent to the measurement error which was estimated by analytical reproducibility. The large differences in diet compositions (e.g. between NM Sey and SM Sey) not being necessarily linked to large differences in $\delta^{15}N$ values*,* the dietary protein quality and quantity cannot be considered as the main source of $\delta^{15}N$ changes. Ménard *et al*. (2007) reported also decreasing $\delta^{15}N$ gradients in the muscle of yellowfin tuna (2.4‰) and swordfish (1.1‰) from the Western Indian Ocean, mainly due to differences in nitrogen dynamics at the base of the food web. This work refers to latitudinal gradient on the Indian Ocean nitrogen fixation mechanisms with a major denitrification zone in the Arabian Sea influencing the Somalian waters, and higher nitrogen fixation processes in the subtropical gyre influencing the Mozambique Channel (Gruber and Sarmiento, 1997; Naqvi *et al.,* 2006). This pattern results in different isotopic signatures of the particulate organic matter which are propagated along the food chain. Spatial differences in the muscle nitrogen isotopic signatures were 1.3-fold higher in skipjack than in yellowfin tuna (Ménard *et al.,* 2007). Two hypothesis may be proposed such as (i) longer residence times for skipjack at least equivalent to the muscle isotopic turn-over (estimated at 3 months in yellowfin, Graham unpublished data) which enables incorporating the regional isotopic signature at the base of the food web, (ii) higher metabolic and turn-over rates allowing for a faster incorporation of environmental isotopic modifications.

In the Western Indian Ocean, the purse-seine fishing effort presents a seasonal pattern (IOTC, 2011) in relation to the migratory behaviour of the tuna species among areas with favourable environment (Fonteneau *et al.,* 2008; Dueri *et al.,* 2012). While, purse-seiners move southwards (towards Mozambique Channel) in April, they move northwards (towards Seychelles islands and Somalia) during the end of the second trimester (IOTC, 2011). Considering a minimum residence time of 2 months in each area and the high metabolic rate in skipjack (Essington, 2003), we could assumed that tissue nitrogen isotopic composition reflected the isotopic composition of its diet. However, isotopic enrichment depends on tissue turnover rates; for example, muscle integrates diet isotopic signatures on a longer time period (months) than do the liver (days/weeks), and might not be in equilibrium with the diet (Tieszen *et al.,* 1983; MacNeil *et al.,* 2006; Madigan *et al.,* 2012). In this sense, variations in $\Delta\delta^{15}N_{M\text{-}L}$ can be related to the fact that tuna do not feed on local prey baseline long enough to reflect the steady-state in muscle. Based on the general fisherman movements (IOTC, 2011), $\Delta\delta^{15}N_{M\text{-}L}$ may reflect migration between isotopically discrete regions that may occur on shorter time scale than the skipjack muscle turn-over rate. Migrations from Seychelles towards Mozambique Channel, an area under the subtropical gyre influence with high nitrogen fixation processes and low $\delta^{15}N$ baseline signatures, might enable liver isotopic values to be diluted resulting in higher $\Delta\delta^{15}N_{M\text{-}L}$ (2.02±0.47‰). The low $\Delta\delta^{15}N_{M\text{-}L}$ observed in fish from Somalian waters (0.41±0.49‰) might respond to the inverse situation. On the other hand, similar $\Delta\delta^{15}N_{M\text{-}L}$ values observed in skipjack captured in Seychelles during NM and SM, could reflect the equilibrium with the diet in both seasons. Then, these tunas could be resident of Seychelles region. Coexistent of migrating tropical tuna together with more resident ones was previously reported in the Eastern Pacific (Hilborn and Sibert, 1988; Popp *et al.,* 2007) and Indian Ocean (Ménard *et al.,* 2007).

The $\delta^{15}N$ signatures measured in muscle skipjack caught in the Mozambique Channel were in accordance with those reported by Ménard *et al.* (In press) for gelatinous organism and small fish in the area and values measured by Graham *et al* (2007) for Pacific small yellowfin (< 45 cm) feeding on planktonic organism. However, our estimations were 1.7 fold lower than the ones observed in yellowfin (< 80 cm) from the Mozambique Channel (Ménard *et al.,* 2007). Hence, female skipjack (L_F = 47±6cm) might occupy a lower trophic level than medium-size yellowfin ($L_F < 80$cm), and would feed mainly on small micronekton in the Mozambique Channel as comforted by SCA results. Nevertheless, further investigations specifically dedicated to the inter-species comparison of tuna isotopic patterns are needed to validate the present assumptions due to annual and seasonal variability of the basal food web isotopic composition (O´Reilly *et al.,* 2002) and to inter-species differences of the trophic discrimination factors and tissue turn-over rates (MacNeil *et al.,* 2006; Madigan *et al.,* 2012).

Relationship between skipjack isotopic composition and spawning activity

To our knowledge, few studies have investigated the impact of fish physiological processes on the carbon and nitrogen isotopic fractionation. Lorrain *et al.* (2002) and Schmidt *et al* (2004) observed changes in isotopic signatures in relation to differences in energy allocation to reproduction instead of diet changes. Schmidt *et al* (2004) predicted that ^{15}N fractionation should decrease with the efficiency of nitrogen deposition (i.e. ratio between protein assimilation and loss). Similarly we suggest that variations in $\delta^{15}N$ values observed in liver and muscle may respond to protein metabolism during the reproductive cycle. The lowest $\delta^{15}N$ values were observed in the liver of spawning capable fish. The protein accumulation due to lipoprotein (e.g vitellogenin) synthesis in the liver (Tyler and Sumpset, 1996) may reduce levels of protein degradation and excretion, and thus, be responsible of the $\delta^{15}N$ decrease *(*Schmidt *et al.*, 2004; Martinez del Rio *et al*., 2009). Protein accumulation may also explain the increase of liver relative weight during gonad maturation observed in skipjack (Grande *et al.,* Submitted*b*). In addition, the accumulation of some amino acids, such as alanine which is part of vitellogenin in fish (De Vlaming *et al.,* 1980; Tyler and Sumpter, 1990), may also contribute to the $\delta^{15}N$ decrease (Schmidt *et al*., 2004). In contrast, muscle $\delta^{15}N$ values increased from immature to spawning capable phase. The higher $\delta^{15}N$ values in spawning capable fish may indicate higher protein degradation to protein synthesis ratio, attributed to the energetic cost of reproduction (Schmidt *et al.,* 2004). Therefore, proteins in muscle may be an energy source for reproduction in skipjack, as was observed in scombrids, i.e. *Sarda sarda* (Zaboukas *et al.,* 2009). In light of this issue, skipjack may rely in muscle energy reserves to fulfil energy requirements during gonad maturation, and thus, not only be dependent of the current energy income as was predicted by Grande *et al*. (submitted*b*).

Conclusions

Peaks in the reproductive activity of skipjack population were related to seasonal increase in food quality and abundance. The indeterminate fecundity and energy allocation strategy type adopted by the species demands a continuous energy supplied through the diet for yolk production throughout the long spawning seasons. Under unfavourable conditions, egg development ceases. $\delta^{15}N$ fluctuations between skipjack reproductive phases gave evidence of an existing relationship between muscle energy stores and reproduction. Protein mobilization from the muscle probably occurs for energy supply to gonad development. Spatiotemporal variations of the nitrogen isotopic composition reflected differences in the isotopic signature at the base of the food web mainly due to the latitudinal denitrification/nitogen fixation gradient existing in the Indian Ocean. Finally, the investigation of $\delta^{15}N$ differences between muscle and liver brought insights on the migration routes of the skipjack between discrete regions in the Western Indian Ocean. To our knowledge, the present work firstly investigates the potential relationship between the reproductive activity, feeding activity and tissues isotopic composition of the skipjack tuna. Further laboratory experiments specifically on protein metabolism during reproduction and amino acids composition should be conducted in order to verify the advanced hypotheses.

10. General discussion

The main objective of a reproductive strategy, which refers to the complex of reproductive traits, is to maximize reproductively active offspring in relation to available energy and lifespan (Wootton, 1998). Exposed to the natural selection, fish has adopted different reproductive tactics which permits to adapt to changeable environments and inherit diverse traits which resulted in a wide range of reproductive strategies (Murua and Saborido-Rey, 2003; Saborido-Rey *et al.,* 2010). Therefore reproductive modes manifested in a species are not linked at random, but respond to the use of energy and allocation rules for growth and reproduction (Wootton, 1998; Saborido-Rey *et al.,* 2010).

Scombrids have diverse reproductive and life history traits (Fromentin and Fonteneau, 2001; Juan-Jordá *et al.*, 2012), and thus variable adaptative response to environmental changes (Winemiller and Rose, 1992; King and McFarlane, 2003; Winemiller, 2005). As mentioned along this dissertation, understanding the relationship between various reproductive traits, maternal attributes and environmental factors allows quantifying the reproductive potential, which may contribute to improve recruitment predictions and effectively quantifying resilience of species to fishing pressure (Tripple, 1999; Marshall *et al.*, 2003; Marshall, 2009). In light of this issue, and considering that skipjack sustains one of the most important fisheries in the world (ISSF, 2012; Arrizabalaga *et al.*, 2012), this work will allowed to improve knowledge on skipjack reproductive dynamics to provide the basis for a sustainable management of the Indian Ocean stock.

The general discussion follows the structure of the PhD thesis. In this section the information of the literature and the acquired experience of the author are combined to gain insights into the mechanism involved in skipjack gamete production.

Oocyte development and fecundity type of the skipjack in the Western Indian Ocean

The fecundity regulation model adopted by a species (i.e. indeterminate or determinate fecundity regulation) is a backbone concept in studies of fish biology and population dynamics (Hunter and Macewicz, 2003; Murua and Saborido-Rey, 2003;

Kjesbu, 2009). It refers to the way in which the oocytes are recruited in the ovary during the maturation and spawning process, and therefore constitute a basic knowledge of the assessment of the fecundity and stock reproductive potential. The distinction between the two fecundity types would determine the selection of fecundity estimation (Murua and Saborido-Rey, 2003; Murua *et al.*, 2003; Kjesbu, 2009) or egg production methods (Armstrong and Witthames, 2012; Bernal *et al.*, 2012). Various works suggested indirectly that the skipjack shows indeterminate fecundity (Cayré and Farrugio, 1986; Stéquert and Ramcharrun, 1995, 1996; Timohina and Romanov, 1996). However, these studies lack of evidences. In order to clarify the fecundity regulation strategy adopted by the species, **Chapter 1** provides and explores various criteria based on previous recommendations (Hunter *et al.*, 1992; Greer Walker *et al.*, 1994; Murua and Saborido-Rey, 2003).

The lines of evidence studied in **Chapter 1** show that skipjack has an asynchronous ovarian organization and indeterminate fecundity type. The previtellogenic oocytes continuously turn into vitellogenic and maturing oocytes during the reproductive season (Murua and Saborido-Rey, 2003). The spawning occurs in several batches when the sea surface temperature is over 24ºC (Schaefer, 2001*a*, 2001*b*, 2001*c*). As observed in the **Chapter 1** this species can be characterized as having an early batch formation (i.e. in terciary vitellogenic stage). If compared with other tuna species, skipjack shows the highest spawning frequency, i.e. 1.2 days (Hunter *et al.*, 1986; Schaefer, 2001*b,* 2001*c*). Therefore, in order to compensate the low spawning interval, the early batch formation must responds to a high oocyte development rate through Vtg3, oocyte maturation and final ovulation stages. In captive specimens this process was observed to occur in 8 h (Kaya *et al.*, 1982). To enable this multiple vitellogenesis cycle during the protracted spawning season, steady recruitment from the pool of previtellogenic oocytes may occur in skipjack as was reported for hake (Korta *et al.*, 2010*a*). However, whereas in hake hepatosomatic index (I_{H}) and lipids in liver decreased with gonad maturation (Dominguez-Petit *et al.*, 2010), in skipjack I_{H} and total lipids content of liver reach the highest values during the vitellogenesis (**Chapter 3**). This indicates a high hepatic activity in fish in spawning capable phase that may sustain the continuous exogenous vitellogenesis and high rate of oocyte maturation (Rinchard and Kestemont, 2003). In this sense, oocyte recruitment may completely overlaps the spawning period and skipjack may lie more towards the extreme indeterminacy in the continuum of determinacy/indeterminacy (Ganias, 2013). As mentioned, reproductive traits are not linked at random, thus the oocyte recruitment pattern in skipjack may be linked to energy allocation strategy for reproduction (i.e. income breeding) (**Chapter 3**). Vitellogenesis has high energetic requirements. During this reproductive phase, major catabolism of lipids occurs to provide metabolic energy for oocyte lipoprotein biosynthesis, and follicles are enlarged mainly from the uptake of lipids and yolk proteins from the plasma. Therefore, the number and/or size of the oocytes are tuned by the available energy resource (Sargent, 1995; Lubzen *et al.*, 2010). Although proteins could partly sustain the oocyte recruitment in skipjack (**Chapter 4**), somatic lipids which constitute the main energy depot in fish (Jobling, 1995; Sargent, 1995) are not reduced during gonad maturation (**Chapter 3**). In this sense, multiple vitellogenic and oocyte maturation cycles during the protracted spawning season (**Chapter 2**) may be carried out mainly at the expense of food intake and whenever the food availability enables it (**Chapter 3 and 4**). Similarly, experiments on batch spawners observed that food availability during spawning had significant effects on the number of batches and the inter-spawning interval (Wootton and Fletcher, 2009; Bapary *et al.*, 2012).

In addition, this oocyte recruitment pattern may explain the high abundance of fish in spawning capable phase along spawning season, but the rare occurrence of fish in actively spawning capable phase with oocytes in GVM or hydration stage during day-light hours (**Chapter 1 and 2**, Hunter *et al.*, 1986; Stéquert and Ramcharrun, 1996; Stéquert *et al.*, 2001). We hypothesized that day time oocyte recruitment into vitellogenesis may occur leading to the evening maturation and spawning (Hunter *et al.*, 1986), as observed in *Encrasicholina purpurea* in tropical waters (Clarke, 1987). Considering that ova development is also asynchronous at population level (**Chapter 2**), daily variance of oocyte diameter distribution could not be accurately evaluated by opportunistic sampling at sea upon purse-seiners. Therefore, a different sampling strategy which enables covering sunset and night time hours and experimental studies are needed to clarify the oocyte growth dynamics and its relationship with energy acquisition and allocation strategy adopted by the species. The lack of clearly recognizable POFs or hydrated oocytes does not permit to give conclusive statements about the oocyte recruitment rate and spawning time. Contrary to our observations, previous works in *Sardina pilchardus* and *Lophius litulon* attributed the early batch formation to slow oocyte development rate (Yoneda *et al.*, 2001; Ganias *et al.*, 2004).

While in those species the size frequency distribution is totally interrupted at Vtg2 stage where the hiatus is established, the oocyte recruitment in skipjack is continuous (**Chapter 1**). Due to the above discussed facts and considering the small size and lipid poor mature oocytes **(Chapter 1 and 3**), the energy cost of a single spawning might be low (Hunter *et al.*, 1986; Schaefer, 2001*b*) and supplied mainly from assimilated food, which permits to support the above described oocyte recruitment rate during the long spawning season, i.e. year around spawning (**Chapter 2**).

As oversimplification, such a mode of oocyte recruitment and fecundity type is common among iteroparous fish (i.e. species with multiple breeding seasons), with protracted spawning seasons (**Chapter 2**) and where oocyte development relies mostly on the food availability **(Chapter 3 and 4)** (Hunter and Leong, 1981; Murua and Saborido-Rey, 2003, Kjesbu, 2009). In general, these species inhabit low latitudes (Pavlov *et al.*, 2009) and offshore environments where resources are patchy on a relative large scale (Winemiller and Rose, 1992, 1993). It enables to increase fecundity avoiding the space limitation imposed by the body cavity (Murua and Saborido-Rey, 2003). Thus this strategy permits to release eggs over longer periods increasing egg dispersal and survivorship of the offsprings in heterogeneous environments (Winemiller and Rose, 1993). As long as the environmental conditions are favourable, reproductive investment is enhanced **(Chapter 2 and 4)** maximizing the probability of successful recruitment (Longhurst and Pauly, 1987; Winemille and Rose, 1992). In contrast, during unproductive periods, egg production is reduced **(Chapter 4)** and oocytes can be resorbed by a down regulation mechanism called atresia (Kjesbu, 2009). In indeterminate fish, it is usual to find a generalized intensity of atresia at the end of the individual spawning season, while a low incidence is observed throughout this period (Hunter and Macewicz, 1985*a*; Hunter *et al.*, 1986; Greer Walker *et al.*, 1994; Murua and Saborido, 2003; Murua *et al.*, 2003). For skipjack, a generalized resorption of vitellogenic oocytes was observed during February and March, coinciding with the end of the season of higher reproductive activity. Stéquert and Ramcharrun (1996) and Stéquert *et al.* (2001) also observed a drop of gonadosomatic index (I_G) and plasma steroid hormones during February. Although February seems to be a suitable month for spawning in terms of prey availability **(Chapter 4)**, size specific spawning and asynchrony at population level could induce some fish to enter in regressing phase earlier in the season **(Chapter 2).**

Sex ratio, reproductive timing and batch fecundity of the skipjack tuna in the Western Indian Ocean

Assessment of the stock reproductive potential requires evaluating fluctuations in the reproductive parameters and understanding underlying mechanisms. The maturation together with the growth are the most important traits concerning fish life history strategy (Saborido-Rey and Kjesbu, 2005). Both respond to a feedback mechanism in which the energy is shared with maintenance (Stearns, 1992; Saborido-Rey and Kjesbu, 2005). The amount of energy allocated to these traits would be dependent on genetic, physiological or environmental factors and would enable fish to maximize the offspring production and survival during fish lifetime in a given environment (Stearns, 1992; Wootton, 1998). Generally, specimens experiencing high mortality rates evolved fast life histories (fast-growing and early maturation) to reproduce before dying (Winemiller and Rose, 1982; Juan-Jordá *et al*., 2012). The early maturation is a common characteristic among tropical tuna species (Juan-Jordá *et al*., 2012), but skipjack tuna, particularly, presents the smallest length at 50% of maturity (i.e. 40 cm), followed by *Eutynnus lineatus* (i.e. 47 cm; Schaefer, 1987). Physiological constraints which limit the maximum body size of specimens in tropical waters (Barkley *et al*., 1978) and the high mortality rate supported by the species (Adam, 1999; Eveson, 2011) might induce fish to select for an increase in reproductive effort and adopt a fast life history, i.e. small size, rapid growth and early maturation (Wootton, 1998; Schaefer, 2001*b*; Juan-Jordá *et al*., 2012). Therefore, when fish overcome threshold levels for growth or energy stores, there is a strong selection pressure for individuals to initiate maturation (Schaefer, 2001*b*, 2001*c*). The diversification of surplus production from the soma to invest in reproduction affect negatively the growth rate (Roff, 1983), which was observed to be reduced above 45 cm in skipjack (Eveson *et al*., 2012).

Tunas do not manifest sexual dimorphism in growth and longevity (Vilela, 1990; Schaefer, 2001*b,* 2001*c*; Juan-Jordá *et al.,* 2012). However, males have been observed to reach larger maximum size in several tuna species (e.g. *Thunnus albacares*, *Thunnus alalunga, Thunnus alalunga, Thunnus obesus, Euthynnus lineatus;* Schaefer, 2001*b*; Schaefer *et al*., 2005; Juan-Jordá *et al*., 2012), including the skipjack *Katsuwonus* *pelamis* (Raju, 1964; Timohina and Romanov, 1986; Stéquert and Ramcharrun, 1996). In the present study, despite an estimated sex ratio of 1:1, the number of large-size males was higher than the one of females. It has been suggested that females are exposed to higher mortality rates than males (Schaefer, 2001*b,* 2001*c*; Juan-Jordá *et al.*, 2012). Indeed, spawning has a higher energetic cost for females than for males (Rijnsdorp and Witthames, 2005), and spawning stress could induce higher mortality rate among older females (Schaefer, 2001*b*).

Iteroparous fish should reproduce when the environmental conditions for larvae development are suitable (Wootton, 1998; Wright and Tripple, 2009), and when fish have enough surplus energy to support the cost of reproduction (Lambert *et al.*, 2000; Saborido and Kjesbu, 2005). Seasonality is more evident in high latitudes where production cycles are shorter and timing is mainly induced by annual cycles of daily photoperiod and temperature (Bye, 1984; Pankhurst and Porter, 2003). In tropical regions with low photoperiod and temperature changes, food availability might act as an environmental cue determinant for fish reproductive success in suitable environmental conditions (Bye, 1984; Wootton, 1998; Bapary *et al.*, 2012). Skipjack shows year around spawning in equatorial regions, when surface water temperature is over 24ºC (Schaefer, 2001*a,* 2001*b,* 2001*c*; Stéquert *et al.,* 2001). As observed by Timohina and Romanov (1996), Stéquert and Ramcharrun (1996) and Stéquert *et al.* (2001), we found that around 70% of the mature population was in spawning capable phase along the sampling period, with phases of more intense sexual activity. As hypothesised for tropical fish *Chrysiptera cyanea* (Bapary *et al.*, 2012), *Euthynnus lineatus* (Schaefer, 1987) and also for skipjack (Stéquert and Rancharum, 1996)*,* the later might evolve to more actively reproduce during monsoon events or in response to mesoescale eddies when water productivity is high (**Chapter 3 and 4**). This spawning strategy also ensures larvae survival, which hatch and externally feed in 3 days after fertilization (Ueyanagi *et al.*, 1974; Matsumoto *et al.*, 1984).

In addition, as observed in **Chapter 2** individual spawning periods can be variable (Wright and Tripple, 2009*).* Asynchrony at spawning is eventually occurring in fish inhabiting tropical and subtropical waters and exhibiting long spawning seasons (Lowerre-Barbieri *et al*., 2011*a*), e.g. hake (Murua and Motos, 2006; Dominguez-Petit *et al*., 2010; Korta *et al.,* 2010*b*). As discussed in **Chapter 2**, the seasonal variability of condition indices (condition factor *K*, I_H and I_G) indicated a continuous recruitment to the spawning population. A high variability in I_G and fecundity has been observed for a given size class in skipjack (Cayré and Farrugio, 1986; Goldberg and Au 1986; Timohina and Romanov, 1986; Stéquert and Rancharrum, 1995) suggesting a reduction of egg production along the spawning season (Cayré and Farrugio, 1986). The F_B and egg size generally diminish during the course of the spawning (Kjesbu *et a*l., 1996; Marteinsdottir and Beggs, 2002). As such, the stages at which fish was caught (i.e. at the beginning or at the end of the sequence of egg batch production) might have major importance on the batch fecundity or egg size and hence I_G value (Wright and Tripple, 2009*;* Korta *et al*., 2010*b*).

As an income breeder (**Chapter 3**), the highest reproductive activity of the skipjack occured during periods of food forage availability increase (**Chapter 4**); however, the largest skipjack did not show the highest reproductive investment. According to the literature, older and larger fish with higher body condition spawn earlier in the season and for longer duration than smaller fish (Kjesbu *et al*., 1996; Saborido-Rey and Kjesbu, 2005; Wright and Tripple, 2009); on the contrary, Morgan (2003) reported longer spawning periods for the youngest specimens of *Hipploglossoides platessoides*. In skipjack, larger females did not accumulate more energy (**Chapter 3**), and the gonad relative size and fecundity were not directly related to L_F while the former was positively related to I_H (**Chapter 2**). Although previous works observed a positive relationship between fecundity and skipjack size, fecundity seems to be highly variable in fishes of similar size range (Cayré 1981; Cayré and Farrugio, 1986; Golberg and Au, 1986). Unlike the good adjustment observed by Stéquert and Ramcharrun (1995) in the Western Indian Ocean, usually the variability explained is low: e.g. <30% in the Western Atlantic (Goldberg and Au., 1986). In the present study, L_F only explained 10% of the variability of batch fecundity and the highest batch fecundity indices were observed for medium size classes. By plotting skipjack relative batch fecundity and L_F, the highest values were also attributed to medium size classes, as observed by Cayré and Farrugio (1986) in the Eastern Atlantic. Moreover, I_G did not increase with L_F in skipjack caught in the Western and Eastern Atlantic Ocean (Cayré and Farrugio, 1996; Goldberg and Au, 1986) as found in the Western Indian Ocean (**Chapter 2)**. To our knowledge, very few studies have highlighted such an independency between fish size and reproductive capacity (batch fecundity mainly), e.g. the ballyhoo *Hemiranphus* *brasiliensis* (McBride and Thurman,, 2003) and the European hake *Merluccius merluccius* (Murua and Motos, 2006). As mentioned in **Chapter 2**, reproductive capacity could be linked to the area and/or season (Cayré and Farrugio, 1986; Stéquert and Rancharrum, 1995), or to the nutritional state of the fish, i.e. the skipjack feeding success. However, why it is not positively related to maternal size? Skipjack growth is described by a two stanza growth model indicating a rapid growth up to 45 cm, followed by a slower growth phase (Eveson *et al.*, 2012). The relationship between fish size and gonad relative weight could thus be affected by periods with different weight-length relationships along fish life-time (Wootton, 1998). However, it does not explain the positive relationship observed between I_G and I_H. A first hypothesis would be that variations on the reproductive investment and fecundity may respond to compensatory mechanism between growth and reproduction and current and future reproduction (Stearns, 1992; Wootton, 1998; Saborido-Rey and Kjesbu 2005). In teleosts, reproductive traits are modified to compensate for high adult mortality, both natural and anthropogenic due to fishing. Eveson (2011) estimated U-shape natural mortality rate for skipjack showing high rate at age 1 and 4+ while lower values were observed in between. This increase of adult mortality rate might select for increasing in reproductive effort early in life. The associated cost of reproduction might in turn induce a decrease in growth, future fecundity and survival (Stearns, 1992; Wootton, 1998). In addition, fishing is known to increase the mortality rate in the older age classes, as well as to reduce the intra-specific competition leading to an individual faster growth, an earlier maturation and an increase of the reproductive effort at age (Reznick, 1993; Law, 2000; Rochet *et al.*, 2000; Rochet, 2009). Studies in North Sea plaice (Horwood *et al.*, 1986) and sole (Rijnsdorp *et al.*, 1991) observed that fecundity at maturity increased under intense fishing conditions. Similarly, Rochet *et al.* (2000) predicted an increased fecundity at maturity in Peciformes, Pleuronectiformes and Gadiformes in compensation to fishing pressure. We wonder if the high reproductive investment observed during the first spawning year (**Chapter 2)** might reflect such a compensatory mechanism in response to high mortality rate, both natural and due to fishing, among large fish. In this case, it might lead to an additional associated energetic cost and impact on subsequent spawning events, responding to the trade-off between current and future reproduction well known in iteroparous fish (Reznick and Yang, 1993; Kjesbu *et al.,* 1996; Heino, 1998; Saborido-Rey and Kjesbu, 2005). On the other hand, reproductive investment differences between medium and large fishes (**Chapter 2**) could result from an artefact in the data interpretation: As spawning season progresses, the batch fecundity and egg size are reduced (Kjesbu *et al.*, 1996; Murua and Motos, 2006; Korta et al., 2010*b*). As such, responding to the general assumption of a positive relationship between fish size and energy investment to reproduction (Saborido-Rey and Kjesbu, 2005; Kjesbu, 2009), at fishing time larger fish may have lower energy reserves and reproductive capacity due to an high reproductive investment carried out along the long individual spawning season. Consequently, as mentioned, individual fecundity would depend on the season in which the fish was caught, i.e., at the beginning or at the end of the sequence of egg batch production (Wright and Tripple, 2009). As such, the low I_G and batch fecundity of larger fish might reflect advanced stage in the individual spawning season. This could also explain the observed high batch fecundity variability in fishes of similar size. Experiments on captive tunas would help to understand energy allocation rules during fish life-time and to evaluate variations in energy investment to reproduction.

Lipids in female skipjack tuna from the Western Indian Ocean: Influence of the reproductive cycle and the fishing environment

The suite of life history traits results from the energy allocation trade off between growth, maintenance and reproduction (Winemiller and Rose, 1982; Wootton, 1998). As such, three primary strategies are distinguished: opportunistic (i.e. small, rapidly maturing and short live fishes), periodic (i.e. large, late maturing, highly fecund with longer life-span) and equilibrium (indeterminate sizes, moderate to late maturation, low fecundity, large eggs and parental care). However, a common requirement for resource allocation is the storage which occurs as an anticipate response to predictable changes that could affect fish survival or reproductive success (Reiznick and Braun, 1987; Bonnet *et al.*, 1998). Accounting for energy acquisition and allocation pattern to reproduction, the species are classified as capital or income breeders (Drent and Dant, 1980; Stearns, 1989). Capital breeders provision offspring using energy stores accumulated earlier in the season mainly as lipid compounds (Sargent, 1995; Wootton, 1998), whereas, in income breeders, reproduction is financed by energy gained concurrently (i.e. current feeding). Capital breeding permits to shift the timing of reproduction and periods when resources become available for adults (Jonsson, 1997), a typical reproductive strategy of temperate and/or migratory species. Fish accumulate lipids during the productive season (i.e. spring-summer) to sustain metabolic costs associated with (i) fish over-winter maintenance, (ii) swimming activity during migrations towards spawning areas, and (iii) gonad maturation known to be the main energy drain in fish (Sargent, 1995; Wootton, 1998). The importance of such energy stores changes with latitude (Garvey and Marschall, 2003). In tropical and sub-tropical pelagic waters, seasonal changes in photoperiod, temperature and food availability are not so marked (Bye, 1984; Bapary *et al.*, 2012), therefore allocation to fat reserves is not primordial (Garvey and Marschall, 2003). Pre- or post-breeding costs will influence the selection of the breeding tactic to evolve (Jonsson, 1997). As mentioned, capital breeding strategy releases the organism to forage when reproduce and allows matching the seasonal environmental peaks for offspring fitness and spawning. However, it involves a surplus foraging prior to spawning associated with a survival cost due to a higher predation risk, and a metabolic cost due to storing itself. In addition, the energy gain acquired from feeding is reduced. As such, in capital breeders, the pre-breeding costs must be lower than the benefits obtained from storing (described in detail in Jonsson, 1997). In contrast, income breeders avoid the above mentioned pre-breeding costs adopting a more efficient energy transfer, but constraining reproductive events to periods of increased food availability. In addition, by enhancing their metabolic activity to compensate their high foraging activity during reproduction, their vulnerability to environmental changes and the risk of diseases might increase (Jonsson, 1997; Garvey and Marshall, 2003; Varpe *et al.*, 2009).

Considering storage cycles as a fraction of energy allocation rules, differences in energy balance between reserves and concurrent supplies are correlated with the Opportunistic-Periodic-Equilibrium life history triangle (Winemille and Rose, 1982; Saborido-Rey *et al.*, 2010). For instance, reproduction in opportunistic species is commonly financed by current incomes (Rijnsdorp and Witthames, 2005). These strategies represent the ends of a continuum while mixing modes is common among fish species (Alonso-Fernandez and Saborido-Rey., 2012), and the selection will determine the organism sensibility to short term environmental fluctuation (Winemiller and Rose, 1982; King and McFarlane, 2003). In addition, the relative importance of different tissues (i.e. muscle, liver and adipose tissue) for lipid storage and mobilization linked to reproduction is species-specific. Studies on fish reproductive biology and population dynamics are therefore of primary importance to understand when and how the resources to reproduction are acquired in order to predict possible changes in the outputs and evaluate the suitability of simple condition indexes to be used as indicators of reproductive potential (Lambert *et al.*, 2003; Marshall *et al.*, 2003). As such, the scope of the **Chapter 3** was to elucidate the surplus energy allocation strategy in skipjack during the reproductive cycle.

As observed in **Chapter 2**, skipjack seem to maximize the amount of energy invested to reproduction. The decrease of the energy allocated to to growth was attributed to the onset of maturation (Eveson *et al.*, 2012) which might lead to an increase of the energy investment to ova development and/or fat reserves (Garvey and Marschall, 2003). According to results in **Chapter 3**, lipids were not accumulated in skipjack somatic tissues to be invested in forthcoming reproductive events. At least, ovarian growth and maturation occurred without any depletion of hepatic and white muscle fat stores. The increasing lipid content and relative weight of liver together with the gonad development may respond to transitory accumulation of lipids in the liver and high vitellogenic activity of this tissue throught the ova development cycle. Dietary lipids are digested and absorbed into the intestines, then transported by lipoproteins through the circulatory system to the liver and finally to the adipose and peripheral tissue depots (Tocher, 2003). During vitellogenesis, estradiol 17-β (E2) promotes hepatic vitellogenin proteins (Vtgs, mainly containing phospholipids) and very low density lipoproteins (VLDL, mainly containing neutral lipids) synthesis (which are massively transported *via* bloodstream to the oocytes. As such, lipids are accumulated in the oocyte as yolk and oil globules leading to the oocyte growth (Tyler and Sumpset, 1996; Wiegand, 1996; Babin *et al.*, 2007). When oocyte recruitment occurs continuously during the spawning season (i.e. indeterminate fecundity) and provisioned by current incomes (i.e. income breeding), the hepatic vitellogenic activity remains intense (Rinchard and Kestemont, 2003) but not at the expense of other energy depots (Perez *et al.*, 2007). Therefore, liver may buffer the discontinuous effect of feeding allowing for continuous deposition of yolk in developing oocytes during the long spawning seasons of indeterminate fish, as the skipjack tuna (Bonnet *et al.,* 1998; Rinchard and Kestemont, 2003). The positive relationship between I_G and $I_{H,}$ and between gonad lipid content and I_H observed in skipjack may respond to the above described energy flow. When evaluating the effect of somatic energy reserves on the relative number of developing oocytes and relative batch fecundity, we did not observe any relationship between oocyte number and lipid content in the liver or muscle. Estimates of the egg production were uniquely related to lipids in the ova. The absence of a relationship between reproductive outputs and energy reserves is characteristic of income breeders (Stearns, 1992). However, as stated by Alonso-Fernandez (2011) and observed in cultured fish experiments (Hislop *et al.*, 1978; Cerdá *et al.*, 1994; Lambert *et al.*, 2000; Izquierdo *et al.*, 2001), female energetic status might be positively relate to egg quality and thus might affect the reproductive potential.

However to which extent the skipjack reproduction depends purely on incomes is unknown. On the one hand, the rate at which the flow of materials or energy acquired, processed and utilized occurs can obscure the existing relations (Stearns, 1992). On the other hand, the role of other storage fractions (i.e., adipose tissue and red muscle), and the analysis of specimens during the sexual resting period remain unexplored to fully understand the skipjack energy allocation strategy. In addition, other macronutrients such as proteins also used as fuel (Love, 1970; Jobling, 1995) were not directly considered in this work. In this sense, **Chapter 4** provides evidence of a relationship between variability in the ratio between protein degradation and synthesis in somatic tissues and gonad maturation (Schmidt *et al.*, 2004; Martinez del Rio *et al.*, 2009).

The income breeding strategy together with the high mass-specific metabolic rate, digestion and evacuation rate (Magnuson, 1969; Olson and Boggs, 1986; Korsmeyer and Deward, 2001; Essington, 2003) might favor an efficient utilization of the resource whitout any associated energetic cost as for capital breeders (Jonsson, 1997). According to Jonsson (1997), income breedeing permits to maximize the reproductive conversion efficiency, i.e. unit of offspring output per unit food input. As previously mentioned along the dissertation, the income breeding is part of a suite of traits adopted to maximize the offspring production during the lifetime (Wootton, 1984; Saborido-Rey *et al.*, 2010). As common fact among species inhabiting tropical waters with protracted spawning season (**Chapter 2**), skipjack has adopted an asynchronous oocyte development and indeterminate fecundity (**Chapter 1**) in which oocyte growth seems to be modulated by feeding success (**Chapter 3**) (Fig 10.1). This strategy permits to maintain a high investment in reproduction when resources are available (Winemiller and Rose, 1993; Kjesbu, 2009; Dominguez-Petit, *et al.*, 2010, Saborido-Rey *et al.*, 2010; Alonso-Fernandez, 2011). If compared with other species, the opposite complex of traits is the one adopted by the capelin (*Mallatus villosus*) inhabiting high latitudes. This is a facultative semelparous species (generally spawning ones in it lifetime) with a synchronous oocyte development and determinate fecundity (Fig 10.1). The potential number of eggs to be spawned is developed at the expense of the energy reserves, i.e. capital breeding (Forberg, 1982; Henderson *et al.*, 1984; Christiansen *et al.*, 2008). Between these two extreme modes, a large diversity of reproductive strategies can be found among fishes (Murua and Saborido-Rey, 2003). For instance, cod (*Gadus morhua*) and pouting (*Trisopterus lucus*) both show determinate fecundity. While in cod the ovarian development is group-synchronous and is financed by previous energy stores (Kjesbu *et al.*, 1991; Kjesbu *et al.*, 1996; Murua and Saborido-Rey, 2003), pouting shows asynchronous oocyte development and is able to compensate inadequate reserves with feeding during the spawning season (Alonso-Fernandez and Saborido-Rey, 2012) (Fig. 10.1).

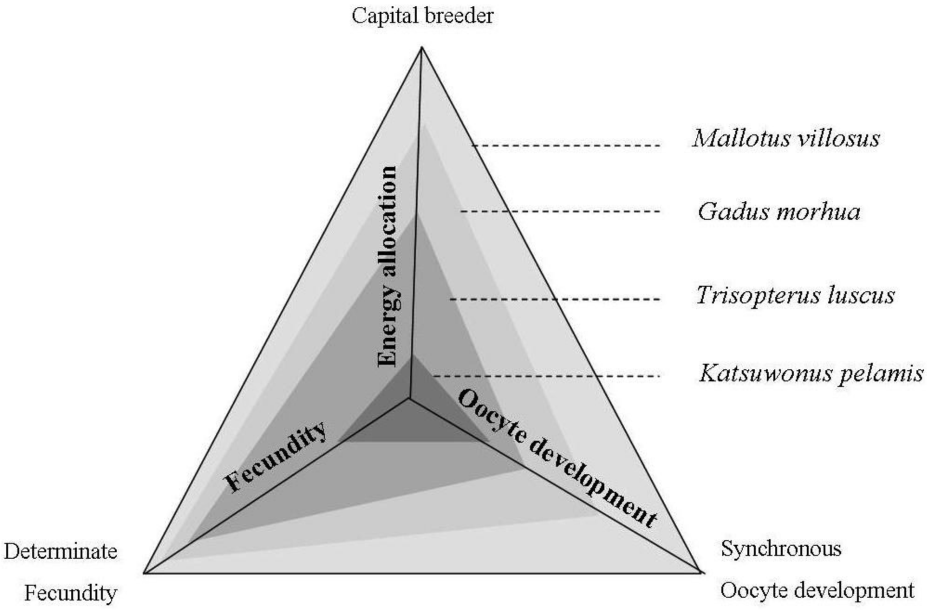

Fig. 10.1. Conceptual relationships between various reproductive modes. The axis represents the continuum of each strategy: oocyte recruitment (asynchronous *vs*. synchronous); Fecundity type (determinate *vs*. indeterminate) and use of surplus energy (capital *vs*. income breeder). Different triangles represent the combination of the suit of traits adopted by each species.

In income breeders, differences in foraging success during the spawning season determine the nutritional status and reproductive potential of specimens. Results in **Chapter 3** indicated significant differences in gonad and liver lipid concentrations of skipjack in spawning capable phase among fishing environments. While feeding success and food quality seem higher during the NM in Seychelles and IM in Mozambique Channel (**Chapter 4**), the highest lipid content was observed in specimens caught in free schools in the IM in Mozambique Channel. Therefore, differences in lipid composition appear to be affected by the fact that the specimens were captured in free schools. This could be justified by the low feeding success observed generally in FADs (**Chapter 4**) and high foraging in free schools (Ménard *et al.*, 2000; Potier *et al.*, 2001; Jaquemet *et al.*, 2011). However, the extent to which these changes in broodstock´s lipid composition could induce changes in energy allocation to reproduction and alter the ovarian growth in skipjack remains unknown. Restricting feeding during oocyte recruitment can slow down oocyte growth, delay start of the spawning, compromise the success of maturation in first-time spawners (Bromley *et al.*, 2000), decrease the egg number, size and quality (Hislop *et al.*, 1978; Kjesbu *et al.*, 1991; Kjesbu *et al.,* 1998), increase the rate of atresia (Ma *et al.,* 1998) and induce the skip spawning (Rideout *et al.*, 2005; Rideout and Tomkiewicz, 2011). However, fecundity is not always equally sensitive to food deprivation (Wootton, 1998). Therefore, before any valuation on the possible effect of FADs on the reproductive potential of the skipjack, various factors should be considered, such as the time of exposure to the low rations (Harel *et al.*, 1994; Bapary *et al.*, 2012), the recovery rate of the species (Bapary *et al.*, 2012), the critical energy point below which the fecundity starts to fall (Kjesbu, 2009) and the priority that is given to the ovarian growth over somatic growth (Wootton. 1998). For instance, the time of exposure to the low rations needed to observe changes in egg and larval quality in *Sparus aurata,* a species characterized by similar fecundity type and energy allocation strategy than skipjack, was of 10 days (Harel *et al.,* 1994). In tropical damselfish, 15 days were needed to observe the disappearance of vitellogenic oocytes (Bapary *et al.*, 2012). When these fish were re-fed, vitellogenic oocytes re-appeared in 30 days (Bapary *et al.*, 2012). It is thought that skipjack commonly do not remain down a FAD more than 2 days and forms cohesive monospecific schools during the day while at night the associations are diffuse and feeding events might occur (Schaefer and Fuller, 2005). Therefore, the reduction on the reproductive potential caused by the associated behaviour remains improbable. On the other hand, while some fishes give priority to egg production at low rations (Wootton, 1977) and conserve oocyte composition (Silversand *et al.*, 1995), a rapid reduction is observed in others (Bapary *et al.*, 2012). Therefore cultured experiments are indispensable to understand insights into the relationship between broodstock diet, tissue lipid composition and egg production. In addition, further information about the duration of FAD-association ("residence times") of skipjack tuna as well as the motivations of such an aggregative behaviour would be determinant factors to better investigate the potential effect of FADs on tuna reproductive potential.

Link between the reproductive activity, feeding strategy and isotopic composition of the skipjack in the Western Indian Ocean

Organism can be monitored as input-output systems in which inputs are constitute by the food resource and outputs by the progeny (Wootton, 1998). Some of the control rules involved in this system have been analyzed in the **Chapter 1, 2** and **3**. However, feeding remains as one of the most important factor influencing reproduction (Wootton, 1998). In tropical and subtropical environments where photoperiod and temperature are fairly constant, food availability could be one of the main factors responsible for the mediation of the reproductive cycle (Bye, 1984; Bapary *et al.,* 2012). In these conditions, the later phase of gametogenesis may be synchronized by environmental changes such as monsoons (Bye, 1984; Bapary *et al*., 2012) in which an increase of nutrients is followed by a burst of productivity (Roger, 1994*b*; Veldhuis *et al*., 1997; Schott and McCreary, 2001; Schott *et al*., 2002; Wieggert *et al*., 2006). In golden perch (Collins and Anderson, 1999) and tropical damselfish (Bapary *et al*., 2012) feeding stimulated the oocyte development in the absence of other environmental stimuli (e.g. temperature and photoperiod). In both species, the change from limited feed to saturated feed conditions induced the appearance of vitellogenic oocytes. Similarly, previous studies on tropical tuna (i.e. yellowfin) observed that forage may explain inter-annual variability in spawning activity (McPherson, 1991; Itano, 2000). Since skipjack ability to complete its reproductive cycle is reliant on adequate levels of nutrition (**Chapter 3**), we hypothesized that ultimately skipjack has evolved to match the spawning season (**Chapter 2**) to periods of high food availability (**Chapter 4**). Thus, the regularly recurring periods of skipjack intense sexual activity during the North-east and South-west monsoons (**Chapter 2;** Stéquert and Ramcharrun, 1996; Stéquert *et al*., 2001) might rely on the increasing food availability (**Chapter 4**). In addition, Mozambique Channel eddies concentrate mikronecton organisms at their periphery (Sabarros *et al*., 2009; Ménard *et al.*, In press), prey aggregations that might enhance feeding opportunities and stimulate ova development in mature skipjack (**Chapter 4**). The energy acquired from the metabolised resources depends on its nutrient composition (i.e. carbohydrates, proteins and lipids) (Olson and Boggs, 1986; Jobling, 1995). Skipjack stomach content analysis revealed a shift from high energetic diets (i.e., fish and cephalopods) in fish caught during the NM in Seychelles and the IM in Mozambique Channel, to lower energetic preys (i.e., crustacea larvae and *Diacavilnia sp.*) in specimens caught during the NM in Somalia and SM in Seychelles. A reduction of the food quality and/or intake ration during oocyte development may reduce fecundity and egg size (Kjesbu *et al.*, 1991; Brooks *et al.*, 1997; Lambert *et al.*, 2000; Izquierdo *et al.*, 2001). Therefore, the highest I_G observed during the NM in Seychelles and the IM in Mozambique Channel (**Chapter 2 and 4**) might be related to higher nutritional status of fish.

The spatiotemporal variations of the isotopic composition ($\delta^{15}N$ and $\delta^{13}C$) of skipjack tissues were not correlated to variations in feeding regimes (**Chapter 4**). Although, $\delta^{15}N$ is commonly used to delineate feeding habits and response to protein intake in terms of amount and quality (Tieszen *et al.*, 1983; Carleton and Martinez del Rio, 2005; Martinez del Rio *et a*l., 2009), in the present study, nitrogen dynamics existing at the base of the food web seem to be the main source of $\delta^{15}N$ changes (Ménard *et al.*, 2007; Popp *et a*l., 2007). As described by Ménard *et al.* (2007), the latitudinal gradient on $\delta^{15}N$ signature in skipjack liver and white muscle resulted from a north-south denitrification/nitrogen fixation gradient (Gruber and Sarmiento, 1997; Schott and McCreary, 2001; Naqvi *et al.*, 2006). As such, the $\delta^{15}N$ of the particulate organic matter (POM) at the base of the food web decrease with latitude and is propagated along the food chain. On the other hand, hepatic tissue is characterised by high isotopic turn over rate reflecting recent input assimilations, while white muscle has slower metabolic activity leading to an integration of the diet isotopic composition on a longer period and frequently might not be in equilibrium with the diet (Tieszen *et al.*, 1983; MacAvoy *et a*l., 2001; MacNeil *et al.,* 2006; Madigan *et al.,* 2012). In this sense, $\Delta^{15}N_{M\text{-}L}$ latitudinal gradient could be related to the fact that skipjack do not feed on local prey baseline long enough to reflect the steady-state in muscle. Therefore, differences in $\Delta^{15}N_{M\text{-}L}$ on fish caught in different regions might reflect migration between areas, which might occur on shorter time scale than the time needed to reflect the isotopic composition of the area in the muscle (**Chapter 4**).

In order to compensate for energetic demands for reproduction, fish usually increase the amount of the resource available by endogenous supply or by changing foraging decisions. The different tactics of energy allocation are described in **Chapter 3** and discussed above**.** As skipjack seems to rely on feeding success to carry out gonad development, it might increase its feeding rate while reproducing in response to increasing energy demands (Jonsson, 1997). This strategy was already described in large tropical pelagic fishes (i.e. yellowfin) in which feeding and spawning activity were observed to occur in the same time-area strata (MacPherson, 1991; Itano, 2000; Bard *et al.*, 2002). In light of this issue, we expected to find more intensive feeding in specimens in spawning capable phase in order to provision the high energy requirements of the vitellogenesis (Babin *et al.*, 2007). We observed that skipjack feeds during the spawning capable phase, but due to the high number of empty stomach (**Chapter 4**) we were unable to corroborate this hypothesis through the stomach content analysis. Since $\delta^{15}N$ increase with diet protein content (Martinez del Rio *et al.,* 2009), it might also reflect changes in fish feeding activity along the reproductive cycle. Indeed, Box *et al.* (2010) observed higher muscle $\delta^{15}N$ signatures in relation to high feeding activity in pre-spawning fish in order to sustain gonad maturation. As such, we expected to find changes in the isotopic composition among reproductive phases when feeding activity was though to be higher in response to higher energetic requirements (i.e. spawning capable phase). Nevertheless, variability of $\delta^{15}N$ is attributed to many factors such as the amino acid composition, isotopic signature, and metabolism and isotope fractionation ($\Delta=\delta_{tissue}- \delta_{diet}$) influenced by body size, growth, protein turn-over rate, nutritional stress, diet composition (i.e. protein quantity and quality) and temperature change which produce changes in metabolism among others (Gannes *et al.*, 1997; Gannes *et al.*, 1998; Martinez del Rio *et al.*, 2009). As such, processes that lead to different fractionation are difficult to elucidate using field data (Gannes *et al.*, 1997). Considering results in **Chapter 4**, we hypothesized that differences in liver and muscle $\delta^{15}N$ could be attributed to tissue specific composition and protein metabolism rather than differences in feeding behaviour. However, detailed amino acid composition and explanation of the mechanisms involved are largely unknown. The evaluation of physiological factors producing changes in the isotopic composition along the reproductive cycle of organism was not under the scope of many works (Lorrain *et al*., 2002; Schmidt *et al*., 2004). Based on the information compiled from the literature, lower $\delta^{15}N$ values in the liver might reflect an increase in the efficiency of nitrogen deposition (i.e. ratio between protein assimilation and loss) due to protein accumulation to enable vitellogenin synthesis (Jobling, 1995; Schmidt *et a*l., 2004). Schmidt *et al.* (2004) observed that trophic amino acids in the digestive gland region (such as alanine) of *Euphasia superba* accumulated and show lower $\delta^{15}N$ values than the ones in the remaining body which was attributed to difference in amino acid metabolism. The alanine is also a common amino acid of fish vitellogenin (De Vlaming *et al.,* 1980; Tyler and Sumpter, 1990). Therefore, we hypothesise that changes in the liver composition caused by the accumulation of yolk protein might partly explain the variations in the isotopic composition during the reproductive cycle. In contrast, we suggest that the higher $\delta^{15}N$ values in the muscle of fish in spawning capable phase could be attributed to higher ratios of protein degradation to protein synthesis, related to the high energetic cost of reproduction (Schmidt *et al*., 2004; Martinez del Rio *et al.,* 2009). As such, skipjack might tend to mobilize muscle protein reserves more readily when building up gonads, in the absence of lipids (**Chapter 3**). Proteins are considered as the dominant chemical compounds in fish reproduction (Kjesbu, 2009) and major constituent of the oocyte (i.e. 80%) (Plack *et al.,* 1971; Sargent, 1995; Wiegand, 1996). Various examples in *Cuplea harengus harengus* (Bradford, 1993), *Mallatus villosus* (Henderson et al., 1984) and *Gadus morhua* (Kjesbu *et al*., 1991) showed how proteins are mobilized from somatic tissues during the reproductive cycle to fulfil increasing energy requirements during the vitellogenesis or synthesis of macromolecules to provision oocyte growth. In this sense, Kjesbu *et al.* (1991) observed a positive relationship between the influx of proteins and the mean vitellogenic oocyte diameter in spawning cod. Among scombrids, protein content was observed to be lower in the muscle of spawning *Sarda sarda* (Zaboukas *et al*., 2006). Therefore, $\delta^{15}N$ differences among reproductive phases in skipjack muscle give evidence of a relationship between energy reserves and reproductive capacity (**Chapter 2 and 4**). In this sense, muscle proteins are likely to be involved in energy supply to financed oocyte recruitment and vitellogenesis in skipjack, which usually become an important energy source after using the lipids (Love, 1970; Jobling, 1995; Sargent, 1995). Therefore, rather than being classified in one extreme of the continuum (i.e. purely income), skipjack might compensate the increasing energy requirements during reproduction with protein reserves from the muscle.

11. Conclusions

- Skipjack in the Western Indian Ocean show an asynchronous oocyte development and indeterminate fecundity. In the ovaries of females in spawning capable phase oocyte diameter distribution is continuous without any gap formation between the primary and secondary growth stages. The recruitment of oocytes into the pool of vitellogenic oocytes is continous during the spawning season (*de novo* vitellogenesis). Finally, when reaching the end of the high reproductive period (i.e. February and March), the number of females in regressing phase (showing ≥ 50% of oocyte in atretic stage) increases.

- The skipjack is characterized as having an early batch formation (i.e. in terciary vitellogenic stage). It might responds to a high oocyte development rate among Vtg3, oocyte maturation and final hydration to compensate the high spawning frequency, i.e. 1.2 days (Hunter *et al.*, 1986). Day time oocyte recruitment into vitellogenesis may occur leading to an evening maturation and spawning. Food availability during spawning may tune the number of batches and the inter-spawning interval.

- The overall sex ratio and by size classes is not significantly different from 1:1.

- The estimated length at which 50% of the female population reached maturity is 39.9 cm and 100% of the females are mature at 56 cm. The shape of the maturation curve indicates that when fish overcome threshold levels for growth or energy stores, individuals are submitted to a strong selection pressure to initiate maturation.

- Around 70% of the mature population is in spawning capable phase along the sampling period. Therefore, skipjack show year around spawning with period of more intense sexual activity mainly during the monsoon events (i.e. North-east and South-east monsoon). However, the high variability of the condition indices suggests a continuous recruitment to the spawning population.

- The reproductive capacity of skipjack is the highest during North-east monsoon and and is positively related to hepatosomatic index. As such, skipjack might increase the energy invested to reproduction when food quality and/or availability is enhanced. In addition, intermediate fish show the highest I_G values suggesting an increase energy investment to reproduction during the first spawning year. However, the high variability on I_G indicates that the stage at which fish was caught (i.e. at the beginning or at the end of the sequence of egg batch production) might have great importance on the I_G value. These hypotheses remain unsolved.

- Lipids are not stored in somatic tissues prior to spawning season in order to be used as fuel provisioning of reproductive expenditures. Skipjack might finance oocyte recruitment mainly by using energy gained concurrently by feeding and, therefore, seems to be oriented towards an income breeding strategy. However, muscle protein reserves are likely to be involved in energy supply during the vitellogenesis and/or provisioning the need for structural biomolecules during oocyte development.

- Skipjack do not feed while associated to Drifting Fish Aggregating Devices, and thus, the associative behaviour may impact on the somatic lipid content which was higher in liver and gonads of fish caught in free swimming schools. However, due to an unsuitable temporal and spatial stratification of the sampling, the present results do not allow to demonstrate a clear effect of FADs on the energetic status and reproductive capacity of the skipjack.

- Responding to the energy allocation strategy (income breeding) adopted by the species, peaks in the reproductive activity of the population are related to seasonal increase in food quality and abundance.

- Rather than reflecting spatiotemporal differences in skipjack diet composition, nitrogen isotopic composition ($\delta^{15}N$) of tuna soft tissues reflects nitrogen dynamics existing at the base of the food web (latitudinal denitrification/nitrogen fixation gradient).

- Variations in the muscle and liver nitrogen isotopic composition ($\delta^{15}N$) during skipjack reproductive cycle might reflect changes in the metabolism in relation to the protein degradation to protein synthesis ratio and amino acid composition.

Thesis

Investigation on the relationship among reproductive traits, maternal attributes and feeding regimes has contribute to understand the reproductive strategy adopted by the tropical skipjack tuna, Katsuwonus pelamis, and elucidate key aspects involved in the appraisal of the reproductive potential of this species inhabiting the Western Indian Ocean.

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13. Annex

Annex 1.

Mean (±standard error) of lipid class concentrations (µg.mg^{-1} on a wet weight basis) in (A) gonads, (B) liver, and (C) muscle of female skipjack tunas collected from the Western Indian Ocean. Females are classified in different reproductive phases according to the most developed oocytes present in the ovary: PG = primary growth; CA = cortical alveolar; Vtg1, Vtg2, and Vtg3 = primary, secondary, and tertiary vitellogenesis, respectively; GVM = germinal vesicle migration oocytes; Reg = ≥ 50% of oocytes in atretic stage; *n* = number of samples; SE-WE = sterol and wax esters; TAG = triacylglycerols; ST = sterols; PL = phospholipids; AMPL = acetone mobile polar lipids; nd = not detected

(A)

Reproductive phase	**Oocyte stage**	***n***	***SE-WE***	***KET***	***TAG***	***ST***	***AMPL***	***PL***
Immature	*PG*	9	0.0±0.3	0.2±0.1	1.3±0.1	1.0±0.1	0.6±0.1	1.8±0.2
Developing	*CA*	10	0.5±0.2	0.6±0.2	1.8±0.2	1.2±0.1	0.4±0.1	1.6±0.2
	Vtg1	11	1.7±0.2	0.9±0.2	2.9±0.4	0.9±0.1	0.5±0.1	1.8±0.1
Spawning capable	*Vtg2*	21	4.4±0.1	1.7±0.3	4.7±0.5	1.2±0.1	0.9±0.1	3.6±0.1
	Vtg3	22	5.1±0.1	1.0±0.2	5.8±0.4	1.3±0.1	0.9±0.1	5.6±0.1
	GVM	26	4.5±0.2	1.1±0.2	7.2±0.6	1.2±0.1	1.0±0.1	4.8±0.1
Regressing	*Reg*	11	3.3±0.2	1.8±0.4	4.1±0.6	1.0±0.1	0.7±0.2	2.4±0.1

(B)

Reproductive phase	**Oocyte stage**	***n***	***SE-WE***	***KET***	***TAG***	***ST***	***AMPL***	***PL***
Immature	*PG*	9	0.4±0.2	2.2±0.5	5.1±1.5	0.7±0.1	1.1±0.2	1.3±0.2
Developing	*CA*	10	1.7±0.2	2.8±0.6	4.9±0.8	0.6±0.1	1.2±0.2	1.7±0.2
	Vtg1	10	1.1±0.2	3.2±0.8	5.2±1.1	0.5±0.1	1.1±0.1	1.7±0.2
Spawning capable	*Vtg2*	21	2.7±0.1	3.6±0.7	6.7±1.4	0.7±0.0	1.5±0.2	3.5±0.1
	Vtg3	20	2.2±0.1	3.6±1.1	6.4±1.0	0.6±0.1	1.1±0.1	2.3±0.1
	GVM	27	2.4±0.2	5.1±0.9	8.7±0.7	0.7±0.1	1.4±0.1	2.5±0.1
Regressing	*Reg*	9	1.1±0.2	4.4±2.2	5.1±0.9	0.5±0.1	1.4±0.2	2.5±0.2

(C)

Reproductive phase	**Oocyte stage**	***n***	***SE-WE***	***KET***	***TAG***	***ST***	***AMPL***	***PL***
Immature	*PG*	10	0.1±0.2	0.5±0.2	1.1±0.2	0.2±0.0	0.6±0.2	1.3±0.2
Developing	*CA*	10	0.1±0.2	0.3±0.1	1.9±0.9	0.2±0.0	0.5±0.1	1.2±0.2
	Vtg1	11	0.1±0.2	0.3±0.1	3.4±1.5	0.2±0.0	0.7±0.3	1.3±0.1
Spawning capable	*Vtg2*	20	0.2±0.1	0.7±0.2	2.2±0.5	0.2±0.0	0.6±0.1	1.6±0.1
	Vtg3	20	0.2±0.1	0.3±0.1	1.1±0.2	0.2±0.1	0.4±0.1	1.7±0.1
	GVM	26	0.2±0.2	0.5±0.1	4.3±1.1	0.2±0.0	0.8±0.1	1.2±0.1
Regressing	*Reg*	10	0.1±0.2	0.7±0.2	2.7±0.8	0.2±0.0	0.6±0.1	1.7±0.2

Annex 2.

Mean (±standard error) of lipid class concentrations (µg.mg^{-1} on a wet weight basis) in (A) gonads, (B) liver, and (C) muscle of female skipjack tunas collected in the Seychelles and Somalia surrounding waters during the North-east monsoon (NM Sey and NM So), in the Mozambique Channel during the inter-monsoon (IM Mz), and the Seychelles surrounding waters during the South-west monsoon (SM Sey). FADs = Fish Aggregating Devices; FSCs = Free Swimming Schools; *n* = number of samples; SE-WE = sterol and wax esters; TAG = triacylglycerols; ST = sterols; PL = phospholipids; AMPL = acetone mobile polar lipids; nd = not detected

(A)

Fishing location and season	**School type**	***n***	***SE-WE***	***KET***	***TAG***	***ST***	***AMPL***	***PL***
NM Sey	FAD	17	3.9±0.2	0.8±0.1	5.0±0.1	1.0±0.1	0.7±0.1	2.3±0.1
NM So	FAD	12	3.4±0.2	0.7±0.2	5.3±0.2	1.1±0.1	1.1±0.1	4.5±0.1
IM Mz	FSC	19	8.0±0.1	1.6±0.1	6.2±0.1	1.7±0.1	0.7±0.1	9.5±0.1
SM Sey	FAD	17	5.1±0.1	0.7±0.1	5.5±0.1	1.1±0.1	1.1±0.1	5.7±0.1

(B)

Fishing location and season	**School type**	***n***	***SE-WE***	***KET***	***TAG***	***ST***	***AMPL***	***PL***
NM Sey	FAD	17	1.8±0.2	3.3±0.1	6.5±0.1	0.9±0.1	1.4±0.1	1.7±0.1
NM So	FAD	12	1.5±0.2	2.0±0.1	5.9±0.2	0.5±0.1	1.0±0.1	2.2±0.1
IM Mz	FSC	19	10.6±0.2	6.3±0.1	9.0±0.2	0.5±0.1	1.4±0.1	6.0±0.1
SM Sey	FAD	17	1.1±0.1	1.3±0.1	4.2±0.1	0.6±0.1	1.1±0.1	1.9±0.1

(C)

Fishing location and season	**School type**	***n***	***SE-WE***	***KET***	***TAG***	***ST***	***AMPL***	***PL***
NM Sey	FAD	17	0.2±0.2	0.7±0.1	4.8±0.2	0.2±0.1	1.0±0.1	0.9±0.1
NM So	FAD	12	0.1±0.2	0.5±0.2	1.7±0.2	0.2±0.1	0.5±0.1	1.0±0.1
IM Mz	FSC	19	0.3±0.1	0.3±0.1	1.1±0.1	0.3±0.1	0.3±0.1	3.6±0.1
SM Sey	FAD	17	0.1±0.2	0.2±0.1	0.9±0.1	0.1±0.1	0.3±0.1	1.2±0.1