

Maternal size, not age, influences egg quality of a wild, protogynous coral reef fish *Plectropomus leopardus*

Alex B. Carter^{1,2,3,4,*}, Alexander G. Carton^{1,2,3}, Mark I. McCormick^{3,5},
Andrew J. Tobin^{1,2}, Ashley J. Williams^{1,2,6}

¹Centre for Sustainable Tropical Fisheries and Aquaculture, ²College of Science, Technology and Engineering, ³College of Marine and Environmental Sciences, ⁴Centre for Tropical Water and Aquatic Research, and ⁵ARC Centre of Excellence for Coral Reef Studies, James Cook University, Cairns, QLD 4870, Australia

⁶Secretariat of the Pacific Community, BPD5-98848, Noumea, New Caledonia

ABSTRACT: Maternal effects are widely known to influence early life-history traits of offspring, including egg and larval quality. We studied the relationships between maternal length, weight, age and hepatosomatic index, with indicators of egg quality (egg size, oil droplet size, total lipid content and lipid classes) of a wild, protogynous reef fish, the common coral trout *Plectropomus leopardus*. Of the egg wet weight, 3% was lipids, with the neutral lipids, triacylglycerol (34% of total lipids) and wax esters (40%), being the main source of lipid energy within the egg. Polar lipids comprised 24% of total lipid content. Egg size and oil droplet size increased positively with maternal weight, and egg size increased positively with maternal length ($p < 0.05$). None of the variation in total lipid or triacylglycerol content was explained by the maternal traits we examined, but the proportion of long-term storage lipid wax esters increased with maternal length and weight ($p < 0.05$). Maternal age had no effect on any indicators of egg quality. Hepatosomatic index influenced sterols, although only with fish weight ($p < 0.01$). Our study highlights the variability in egg characteristics of a protogynous fish in the wild, and provides a greater understanding of variation in maternal traits as indicators of egg quality for teleost fishes. Our results suggest that offspring from larger females may have an advantage during the critical transition to successful exogenous feeding through enhanced provisioning, and that size-truncation through fishing may have negative consequences for recruitment and replenishment of *P. leopardus* populations.

KEY WORDS: Egg size · Great Barrier Reef · Lipid · Maternal effect · Reproduction · Serranidae

Resale or republication not permitted without written consent of the publisher

INTRODUCTION

Maternal effects are an important source of non-genetic (phenotypic) variation within a population and are widely known to influence the reproductive potential of a fished stock (Green 2008). The quantity and quality of eggs is often strongly influenced by the female size and age structure of a population; larger, older females often have greater batch fecundity (Sadovy 1996), spawn more frequently (Lowerre-

Barbieri et al. 2009), have a longer spawning season (Claramunt et al. 2007) and produce better quality eggs and larvae (Berkeley et al. 2004a) than smaller, younger conspecifics. Female body condition may also influence egg quality, with the hepatosomatic index (*HSI*) commonly used as an indicator, because of the liver's ability to store high levels of lipids used in egg production (Berkeley et al. 2004a, Macchi et al. 2013). Maternal effects on egg quality can have important implications for the reproductive potential of

fished stocks because age or size truncation of a population through the removal of larger and older females by fishing can cause a greater reduction in the reproductive potential of that population than just a reduction in mature female biomass (Berkeley et al. 2004b, Venturelli et al. 2009, Hixon et al. 2014).

The size and biochemical composition of the egg is an important maternal contribution in fishes. Egg quality is commonly used to describe the condition of fish eggs, which can have a profound effect on fertilization success, hatching success, larval growth and larval survival (Kjørsvik et al. 1990, Brooks et al. 1997). Fish eggs consist mostly of lipoprotein yolk that contains primarily polar lipids mainly in the form of phospholipids, which is a structural lipid used in catabolism for energy and is rich in polyunsaturated fatty acids, and some neutral lipids (Wiegand 1996). Many marine pelagic fish eggs also contain an oil droplet that consists mostly of neutral lipids (mainly triacylglycerol [TAG], wax ester [WE] and/or steryl ester) that provide the egg with most of its energy (Wiegand 1996, Tocher 2003). These lipids provide an important endogenous energy source for the developing embryos and larvae during the period between fertilization and the onset of exogenous feeding (reviewed by Tocher 2003). Egg quality is, therefore, most frequently estimated using measurements of egg size, oil droplet size and/or egg biochemical composition because larger eggs often indicate greater maternal investment into yolk and neutral lipids (Green 2008). Egg and/or oil droplet size has been positively correlated with fertilization rates, larval size at hatching, larval growth rates and larval resistance to starvation (Moodie et al. 1989, Hutchings 1991, Marteinsdottir & Begg 2002, Berkeley et al. 2004a, McCormick & Gagliano 2008). Egg lipid composition indicates the relative types of energy available to the developing embryo; eggs high in neutral lipids are often positively correlated with larval growth and survival (Berkeley et al. 2004a, Hilton et al. 2008).

Maternal effects on egg quality have been researched extensively, but the majority of studies have focused on temperate fishes, many of which are gonochores (see reviews by Wiegand 1996, Kamler 2005, Green 2008), as opposed to tropical species where hermaphroditism, particularly protogyny, occurs in approximately 50% of fish families (Jennings & Kaiser 1998). Research questions are frequently framed in an aquaculture context where maximizing the number of viable larvae produced by female broodstock is a priority (Migaud et al. 2013). Aquaculture-related studies often use pooled egg samples

rather than individual fish to examine the effects of diet or environment on broodstock egg quality (Brooks et al. 1997) or to compare egg quality between wild and captive spawners (Kjørsvik 1994). Less emphasis has been placed on egg quality of individual wild females (Bachan et al. 2012), and even fewer studies have investigated maternal effects on egg quality for tropical reef fishes (Donelson et al. 2008, Maddams & McCormick 2012). Those studies that have examined maternal effects on egg quality for tropical reef fishes have focused on populations of small species that have limited value in commercial food fisheries and are unlikely to be affected by fisheries or protection within no-take marine reserves (Green & McCormick 2005, McCormick & Gagliano 2008, Maddams & McCormick 2012). Maternal effects on egg quality for exploited protogynous fishes are of particular interest for fisheries management because the implications of the direct removal of large females through fishing may be compounded by the loss of large males through fishing, which can induce female–male sex change at younger sizes and ages (Hawkins & Roberts 2003).

This study provides the first description of relationships between maternal traits and egg quality for a wild, protogynous coral reef fish, the common coral trout *Plectropomus leopardus* (Lacépède, 1802). *P. leopardus* are distributed throughout Indo-Pacific coral reefs, and are a valuable food fish in the tropics (Heemstra & Randall 1993). Commercial fishers in Southeast Asia and along Australia's Great Barrier Reef (GBR) target *P. leopardus* for the lucrative live fish export market, with live fish sent predominantly to Hong Kong and mainland China (Sadovy de Mitcheson et al. 2012). On the GBR, *P. leopardus* is also the major target species for charter and recreational reef line fishers (Mapstone et al. 2008). The International Union for the Conservation of Nature (IUCN) listed *P. leopardus* as 'Near Threatened' in 2004 due to population declines in various countries, primarily from overfishing (Cornish & Kiwi 2004). Fishing for *P. leopardus* on the GBR has been implicated in reducing the size and age at sex change, reducing the mean size and age of spawning females, and reducing population density relative to no-take reserve reefs (Carter et al. 2014a,b, Emslie et al. 2015). If larger and older female *P. leopardus* that are in better condition can produce better quality eggs than smaller and younger females, the removal of the former, either directly by fishing or indirectly by fishing-induced early sex change, may reduce the quality of reproductive output on fished reefs relative to reserves.

The objective of this study was to examine the relationship between maternal traits (length, weight, age, *HSI*), egg morphometrics (egg diameter, oil droplet diameter) and egg biochemical composition (total lipid and lipid class composition) as indicators of egg quality in *P. leopardus*.

MATERIALS AND METHODS

Fish capture and egg collection

Gravid females in imminent spawning condition were collected from inner-shelf reefs located in the central section of the GBR, Queensland, Australia. Bramble Reef was sampled in 2008 (18.42°S, 146.72°E) and the neighbouring John Brewer Reef was sampled in 2009 (18.63°S, 146.05°E) (Fig. 1). Mean monthly water temperature (MMWT) data for each sampling period were collected using the Australian Institute of Marine Science historic data tool for Davies Reef (DAVAWSL2 at 8.4 m reef slope site, Site 2, <http://data.aims.gov.au/aimsrtds/datatool.xhtml>) (Fig. 1). Fishing surveys were conducted in late October 2008 (MMWT = 27.0°C), mid-October 2009 (MMWT = 26.7°C) and mid-November 2009 (MMWT = 27.4°C) and coincided with the new

moon lunar phase when spawning activity peaks for *Plectropomus leopardus* (Samoilys 2000). In 2008, live fish were transported to Orpheus Island Research Station at the end of each fishing trip, and in 2009, live fish were transported to Townsville prior to stripping for eggs in the lab. Gravid females were identified by swollen abdomens and urogenital pores. In 2008, 9 gravid females were successfully stripped of hydrated eggs with a further 28 successfully stripped in 2009. To collect eggs, a firm anterior-to-posterior stripping pressure was applied to the abdomen. Eggs released from the urogenital pore were collected in a sterile 600 ml sample jar whilst ensuring faeces, urine, blood and/or seawater did not contaminate egg samples, and that all eggs collected remained unfertilized. Egg samples from each female were divided in half for analysis of egg size characteristics (morphometry) and egg biochemistry.

Each stripped fish was anaesthetized, fork length (*FL*) measured to the nearest millimeter and weighed (total weight = *W*) to the nearest gram. We were unable to weigh all females due to a malfunction that developed with the scale during the November 2009 sampling period. The liver from each fish was also removed and weighed to determine *HSI* as an indicator of body condition and energy reserves using the formula: $HSI = (\text{liver wet weight}/\text{total female}$

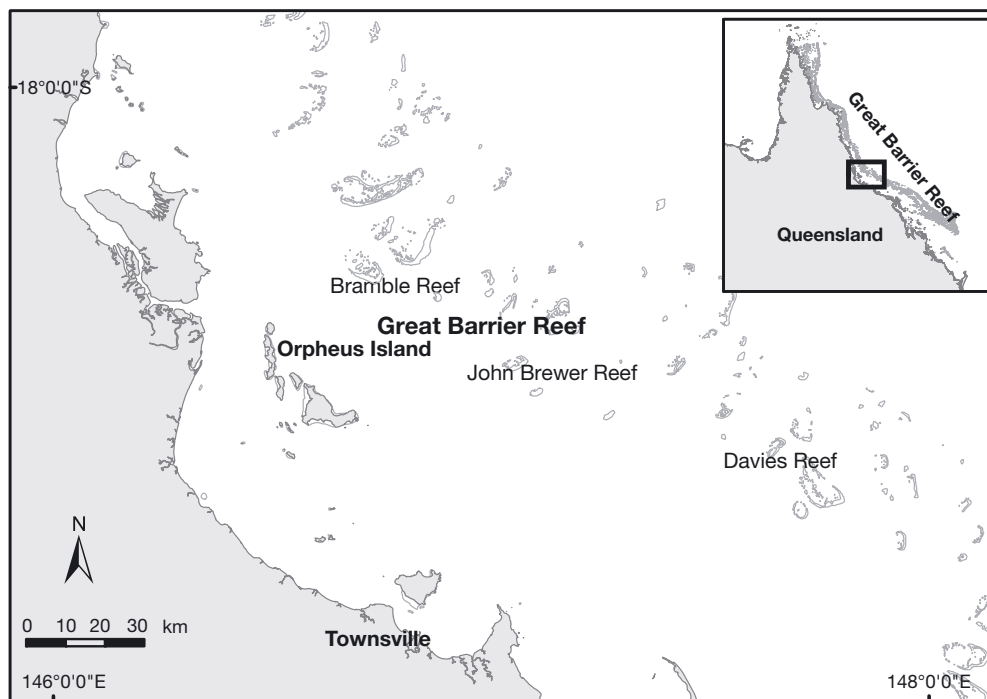


Fig. 1. Reefs where common coral trout *Plectropomus leopardus* were sampled on the central Great Barrier Reef, Australia, to obtain egg quality estimates

wet weight) $\times 100$ (Wootton 1977). Otoliths were removed, dried and stored in envelopes for later aging. Age (A) was estimated by counting annuli in sectioned otoliths using the method described by Ferreira & Russ (1994).

Egg morphometry and lipid composition

P. leopardus eggs contain a single oil droplet (Fig. 2). For egg morphological measurements, fresh samples of whole, hydrated eggs were stored in a 1 ml cryovial with a small amount of seawater and refrigerated for <24 h prior to being photographed using a high-powered digital photo-microscope with a 1 mm scale bar. Photographs were loaded into ImageTool version 3.0 (UTHSCSA), calibrated, and measurements of egg diameter (ED) and oil droplet diameter (ODD) were taken to the nearest 0.01 mm of $n = 30$ randomly selected eggs per female.

For lipid composition analysis, remaining whole, hydrated eggs were distributed into ca. ten 1 ml cryovials immediately after stripping, frozen in liquid nitrogen and stored at -80°C for 14 to 15 months

(2008 samples) and 2 to 3 months (2009 samples) prior to analyses. Eggs were emptied into a 750 ml vacuum flask and rinsed with Milli-Q water for 2 to 3 min whilst under vacuum. Egg samples were filtered on Whatman 25 μm GF/F filters, removed from the filter paper using a clean stainless steel spatula, placed into pre-weighed 2.5 cm diameter glass vials and the wet weights (± 0.01 g) of the egg samples were measured. Egg samples were homogenized for 5 to 10 min using a CAT hand-held homogenizer (X120). Accurate quantities of methanol, chloroform and Milli-Q water (according to the Bligh & Dyer 1959 method) were used separately throughout the homogenization of samples with the intention of cleaning the homogenizer of any tissue residue. After each egg sample was processed, the homogenizer was disassembled and cleaned with Milli-Q water and chloroform.

Total lipid was extracted from each egg sample by the modified Bligh & Dyer (1959) method using a one-phase methanol:chloroform:water solvent mixture (2:1:0.8 v/v/v). Approximately 0.5 g of each egg sample was weighed to the nearest 0.01 g before each sample was extracted. Egg samples and sol-

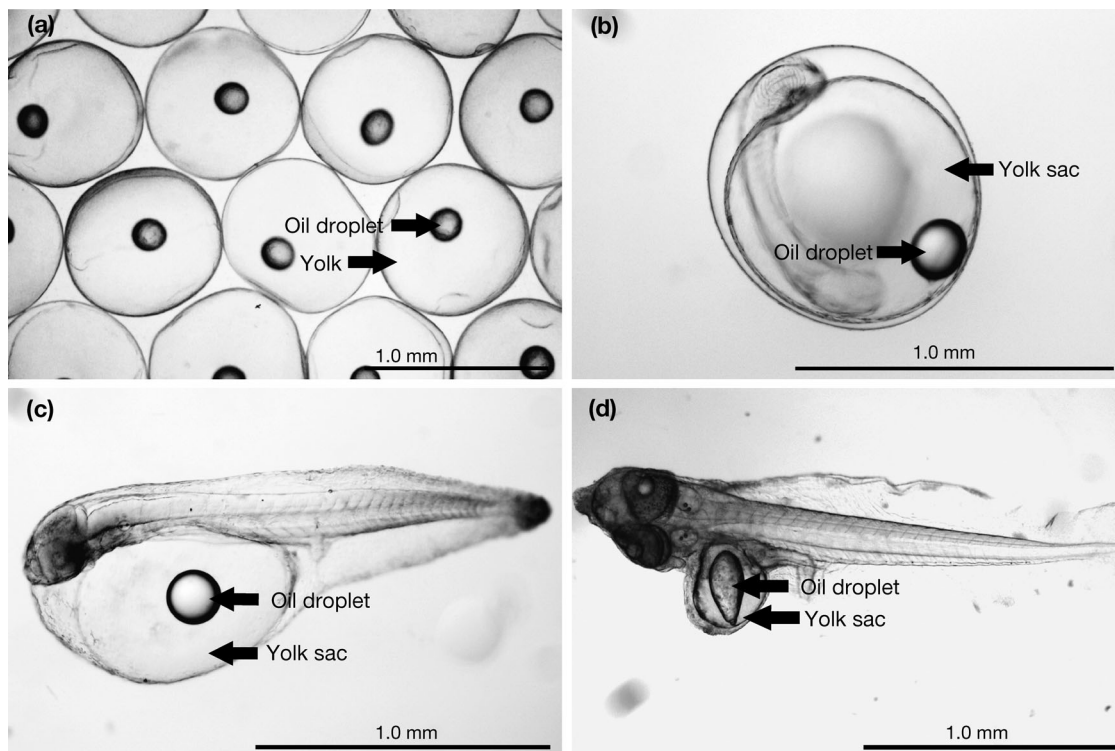


Fig. 2. *Plectropomus leopardus* eggs with oil droplet at varying stages of development: (a) unfertilized eggs; (b) developing embryo, 3 h post-fertilization; (c) newly hatched larva still dependent on endogenous reserves, 3 h post-hatching; (d) larva at approximate onset of exogenous feeding with greatly depleted yolk sac, 43 h post-hatching. Photos: A.B.C.

vents were placed into 250 ml separatory funnels and shaken to form a miscible solution. Each sample was left overnight before breaking phase by the addition of chloroform and water so that the final ratio of solvents was 1:1:0.9. Samples were then left for approximately 4 h to allow sufficient time for the separation of an upper aqueous phase and a lower chloroform phase. Total lipids were recovered from the lower chloroform phase into 250 ml round bottom flasks followed by the removal of chloroform *in vacuo* using a rotary evaporator at $\sim 20^{\circ}\text{C}$. The total lipid extract (TLE) was transferred to chloroform-washed vials and concentrated by application of inert nitrogen gas, and diluted for further analyses. A quantity of 1 ml of chloroform was added to samples so that the final concentration was ~ 10 mg of lipid in the vial. The TLE was weighed to determine total lipid content (%) where 1% lipid content (wet weight) is 10 mg g^{-1} (wet mass).

Lipid class composition of egg samples was determined using an Iatroscan Mark V TH10 thin layer chromatograph (TLC) coupled with a flame ionisation detector (FID) (Nichols et al. 1994). For each sample, the TLE was spotted and developed in a polar solvent system (60:17:0.1 v/v/v hexane:diethyl-ether:acetic acid); samples were also run in a non-polar solvent system (96:4 v/v hexane:ether) to resolve hydrocarbon (mainly squalene, SQ) from WE and diacylglycerol ethers (DAGE) from TAG. All samples were run in duplicate along with standard solutions, which contained known quantities of WE, DAGE, SQ, TAG, free fatty acids (FFA), sterols (ST) and polar lipids (PL). Chromatograms were oven dried for 10 min at 100°C and analysed immediately. Peaks were quantified using DAPA Scientific Software. Total lipid (TL) class content was obtained by summing the individual lipid classes determined using the Iatroscan TLC-FID. TL content of egg samples was also obtained gravimetrically by TLC-FID. TL content reported here was obtained by averaging TL content obtained gravimetrically and independently for all individual samples. TL and lipid class values represent the average of 2 samples, except where one value was considered an outlier and removed when accuracy was $< 80\%$.

Data analysis

Egg samples from both years were pooled due to small sample size and Pearson's product-moment correlation coefficients were used to examine relationships between egg quality indicators TL, WE,

TAG, FFA, ST, PL, ED and ODD. ED and ODD were log-transformed prior to correlation analysis but not for the generalized linear models (GLMs). All statistical analyses were conducted in the statistical software environment R (v.3.0.2) (R Development Core Team 2013).

The effects of maternal traits (factors: *FL*, *W*, *A* and *HSI*) on each indicator of egg quality (response variables: TL, WE, TAG, FFA, ST and PL) were modelled using GLMs with a Gaussian distribution in the lme4 package (Bates et al. 2012). Explanatory variables were tested for collinearity prior to fitting the models using variance inflation factors (VIFs) (Zuur et al. 2009) in the car package (Fox & Weisberg 2009). The VIFs of *W* and *FL* were > 9 , indicating that these factors were highly correlated. When either *W* or *FL* were removed and the VIFs recalculated, all VIFs were < 3 , indicating that the collinearity among variables was within reasonable limits and would not substantially inflate the standard errors of each model's parameter estimates (Zuur et al. 2009). Separate *W* and *FL* models were then run that each included a 3-way interaction with *A* and *HSI*.

To determine the optimal model, a global model was created for each egg quality indicator where all explanatory variables up to 3-way interactions were considered. Sub-model sets of the global model were then generated using the dredge function in the MuMIn package (Barton 2013). MuMIn requires no missing values in the data set, so prior to analysis the *FL*-*W* relationship for *P. leopardus* was estimated and used to generate missing *W* and *HSI* values. A second *W* model was also run without the 11 females for which *W* and *HSI* were estimated to assess the influence of using imputed weight data. The top set of models were defined as those that fell within 2 Akaike's information criterion corrected for small sample sizes (AICc) (Burnham & Anderson 2002) of the top ranked model in the set. The best-fit model was considered to be the simplest model with the lowest AICc (Burnham & Anderson 2002) and was used as a basis to predict the expected values of response variables. An overall test of the best-fit models was conducted by comparing that model to the intercept-only (null) model with a chi-squared test to determine whether the model selected was significantly better at predicting the measure of egg quality. A p-value of < 0.05 indicated there was a relationship between 1 or more of the maternal predictors and the egg quality indicator. Residual plots and quantile-quantile (q-q) plots were examined for each best-fit model for violations of the assumptions of homogeneity of variance and normality.

RESULTS

Egg characteristics

Egg diameter for *Plectropomus leopardus* ranged from 0.80 to 0.92 mm, and *ODD* ranged from 0.18 to 0.20 mm (Fig. 2a, Table 1). Eggs consisted of approximately 3% TL content. The major portion of the egg lipid was composed of neutral lipids, with the short-term storage lipid WE comprising the largest proportion of TL (~40%) followed by the long-term storage lipid TAG (~34%). The next most dominant lipid class was PL (~24%), while ST accounted for <2% of TL. Low levels of FFA were present in all egg samples (<0.5%). No SQ or DAGE were detected in the eggs.

There was a significant positive relationship between TL and TAG ($r = 0.32$), and between PL and FFA ($r = 0.33$) (Table 2). ST were significantly and negatively correlated with TL and WE, and PL were significantly and negatively correlated with TL, WE and TAG (Table 2). ST and PL were positively correlated ($r = 0.76$, $p < 0.001$) as were measurements of *ED* and *ODD* ($r = 0.42$, $p < 0.01$) (Table 2).

Maternal influences on egg quality

A broad range of sizes and ages of female *P. leopardus* were examined for egg quality. These females

ranged from *FL* = 315 to 525 mm, *W* = 200 to 2400 g, and *A* = 3 to 9 yr (Table 1). The relationships between maternal traits and indicators of egg quality were variable. The best-fit models that described most information on each indicator of egg quality generally included terms for either *FL* or *W* (Tables 3 & 4). None of the models selected included *A*, and *HSI* was a significant predictor of ST only in interaction with *W* (Tables 3 & 4).

TL content within the eggs of *P. leopardus* did not vary with any of the maternal traits examined. However, the proportion of lipid classes within the TL of eggs did vary. WE as a proportion of TL was best described by a model that included *FL* or *W* (Table 3). Eggs spawned by large females (defined as *W* = 2000 g or *FL* = 500 mm) contained approximately 15 and 16% more WE, respectively, than eggs spawned by small females (defined as *W* = 500 g or *FL* = 350 mm) (Table 5). ST and PL as a proportion of TL decreased with female *FL* (Fig. 3, Tables 3, 4 & 5). TAG and FFA as a proportion of TL were not influenced by any of the maternal traits examined and remained constant despite variation in *ED* or *ODD* (Tables 2, 3 & 4).

Egg diameter increased with *W* and *FL*, and *ODD* also increased with *W* (Figs. 3 & 4). Eggs spawned by large females were approximately 4% larger than eggs spawned by small females (Table 5). The models with *W* as a predictor of *ODD*, and *FL* as a predictor of *ED*, were selected

Table 1. Minimum and maximum (all sampling periods combined), and mean \pm SE with sample size (n, for each sampling period and for all sampling periods combined) of mature females and egg characteristics for *Plectropomus leopardus* collected from the central Great Barrier Reef (GBR). Percentage values refer to percent of total lipid within the egg unless otherwise stated. *FL*: fork length; *A*: age; *W*: weight; *HSI*: hepatosomatic index; TL: total lipid; WE: wax ester; TAG: triacylglycerol; FFA: free fatty acids; ST: sterols; PL: polar lipids; *ED*: egg diameter; *ODD*: oil droplet diameter

	Minimum All samples	Maximum All samples	Mean \pm SE (n)			
			October 2008	October 2009	November 2009	All samples
Mature females						
<i>FL</i> (mm)	315	525	458 \pm 12 (9)	409 \pm 12 (19)	430 \pm 12 (12)	426 \pm 8 (40)
<i>A</i> (yr)	3.0	9.0	4.4 \pm 0.4 (9)	4.4 \pm 0.4 (18)	4.1 \pm 0.3 (11)	4.3 \pm 0.2 (38)
<i>W</i> (g)	200	2400	1570 \pm 133 (9)	862 \pm 101 (18)	850 \pm 50 (2)	1081 \pm 96 (29)
<i>HSI</i>	0.3	2.7	1.1 \pm 0.1 (9)	1.2 \pm 0.1 (18)	1.1 \pm 0.6 (2)	1.2 \pm 0.1 (29)
Eggs						
TL (% egg wet weight)	1.5	7.0	3.0 \pm 0.6 (9)	3.2 \pm 0.3 (19)	3.2 \pm 0.4 (12)	3.1 \pm 0.2 (40)
WE (%)	29.9	51.1	43.8 \pm 1.6 (9)	38.3 \pm 1.2 (19)	40.4 \pm 1.6 (12)	40.2 \pm 0.9 (40)
TAG (%)	21.1	41.2	31.9 \pm 1.5 (9)	34.8 \pm 1.1 (19)	35.6 \pm 0.9 (12)	34.4 \pm 0.7 (40)
FFA (%)	0.00	0.51	0.06 \pm 0.06 (9)	0.05 \pm 0.03 (19)	0.06 \pm 0.02 (12)	0.06 \pm 0.02 (40)
ST (%)	0.79	2.60	1.5 \pm 0.1 (9)	1.8 \pm 0.1 (19)	1.5 \pm 0.1 (12)	1.6 \pm 0.1 (40)
PL (%)	10.6	34.6	22.7 \pm 1.6 (9)	25.0 \pm 1.6 (19)	22.4 \pm 1.4 (12)	23.7 \pm 0.9 (40)
<i>ED</i> (mm)	0.80	0.92	0.88 \pm 0.01 (9)	0.84 \pm 0.004 (19)	0.83 \pm 0.006 (12)	0.85 \pm 0.004 (40)
<i>ODD</i> (mm)	0.18	0.20	0.19 \pm 0.002 (9)	0.18 \pm 0.001 (19)	0.18 \pm 0.001 (12)	0.19 \pm 0.001 (40)

Table 2. Correlations for egg quality indicators total lipid (TL, proportion of egg wet weight), wax esters (WE, proportion of TL), triacylglycerol (TAG, proportion of TL), free fatty acid (FFA, proportion of TL), sterols (ST, proportion of TL), polar lipids (PL, proportion of TL), egg diameter (*ED*, mm) and oil droplet diameter (*ODD*, mm). Pearson's product-moment correlation coefficients (*r*) are presented in the upper triangle and the 95% CI are presented in the lower triangle. Statistically significant correlations (**p* < 0.05, ***p* < 0.01, ****p* < 0.001) and corresponding CI are in **bold**. *ED* and *ODD* were log-transformed prior to analysis

	TL	WE	TAG	FFA	ST	PL	<i>ED</i>	<i>ODD</i>
TL	–	0.20	0.32*	–0.15	–0.36*	–0.40*	–0.10	–0.0003
WE	–0.12 to 0.48	–	–0.21	–0.17	–0.68***	–0.72***	0.12	0.04
TAG	0.01 to 0.57	–0.49 to 0.11	–	–0.28	–0.28	–0.52***	–0.17	0.05
FFA	–0.44 to 0.17	–0.46 to 0.15	–0.54 to 0.04	–	0.20	0.33*	0.21	–0.05
ST	–0.60 to –0.05	–0.82 to –0.47	–0.54 to 0.04	–0.12 to 0.48	–	0.76***	0.01	–0.13
PL	–0.63 to –0.10	–0.85 to –0.53	–0.72 to –0.25	0.02 to 0.58	0.59 to 0.87	–	0.01	–0.06
<i>ED</i>	–0.40 to 0.22	–0.20 to 0.41	–0.45 to 0.15	–0.11 to 0.49	–0.31 to 0.32	–0.30 to 0.32	–	0.42**
<i>ODD</i>	–0.31 to 0.31	–0.28 to 0.34	–0.26 to 0.36	–0.36 to 0.27	–0.43 to 0.19	–0.37 to 0.25	0.13 to 0.65	–

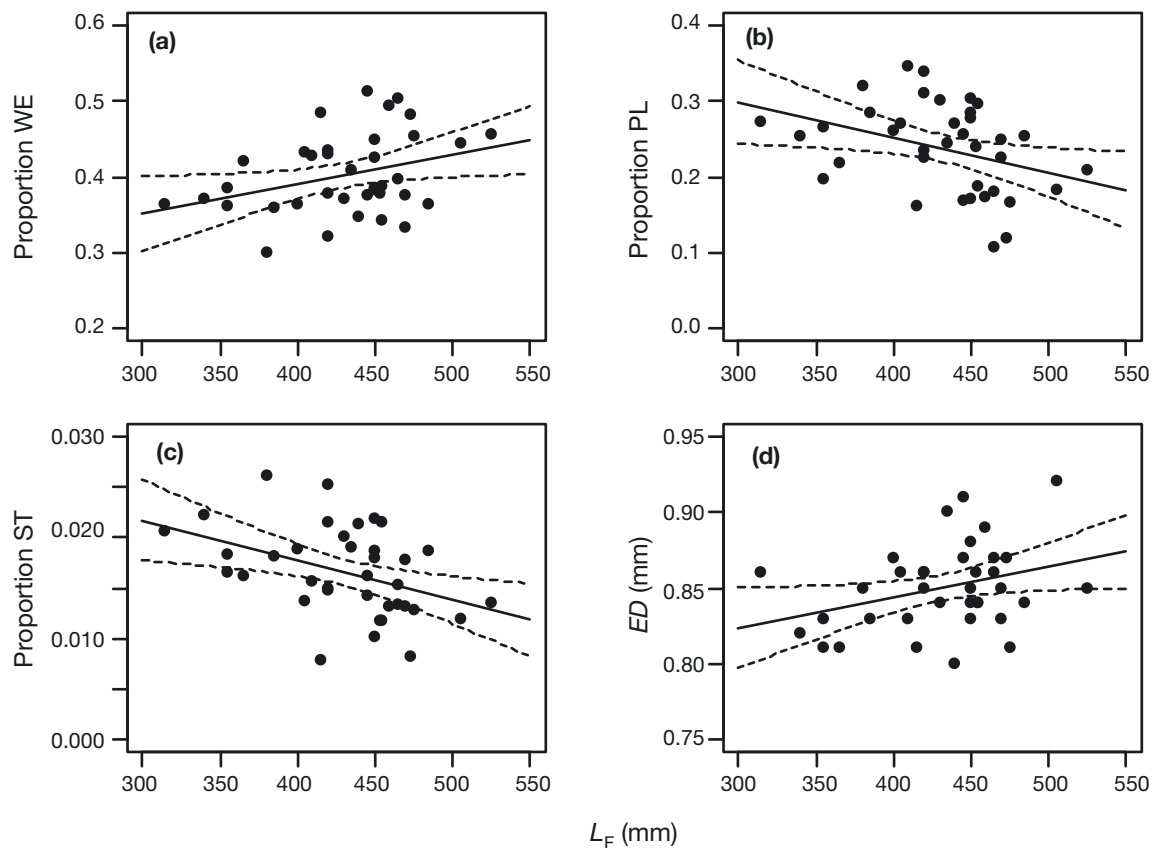


Fig. 3. Relationship ($\pm 95\%$ CI) between (a) fork length (*FL*) and wax esters (WE) as a proportion of total lipid (TL) content; (b) *FL* and polar lipid (PL) as a proportion of TL; (c) *FL* and sterols (ST) as a proportion of TL; and (d) *FL* and egg diameter (*ED*) in *Plectropomus leopardus* eggs. Predictions derived from best-fit models described in Table 3

as the best, despite the null model's inclusion in the top model set (Table 3) because these models were significantly better predictors of *ODD* and *ED* than the respective null models (chi-squared tests, *p* < 0.05).

DISCUSSION

The relationships between maternal traits and egg quality for *Plectropomus leopardus* that we describe from wild populations are the first for a commercially

Table 3. Summary of generalized linear models (GLMs) showing the set of top models ($AIC_c < 2$) predicting the relationship between maternal traits and egg quality indicators for *Plectropomus leopardus*. Two sets of global models were run to avoid collinearity between predictors: (a) weight (W), age (A) and hepatosomatic index (HSI); and (b) fork length (FL), A and HSI . The best model selected for each egg quality indicator is in **bold**. logLik: log likelihood; ΔAIC_c : difference in AIC_c values between model I and the top ranked model of those considered; w : probability that a model is the best model of the set; TL: total lipid; WE: wax ester; TAG: triacylglycerol; FFA: free fatty acids; ST: sterols; PL: polar lipids; ED : egg diameter; ODD : oil droplet diameter; Egg QI: Egg quality indicator

exploited tropical protogynous reef fish. Longer and heavier females invested in larger eggs with greater concentrations of the neutral lipid WE, while maintaining concentrations of the neutral lipid TAG and TL content in the egg, relative to shorter and lighter females. Heavier females also were capable of increasing WE while maintaining PL concentrations in eggs that contained larger oil droplets, compared with eggs produced by lighter females. The positive relationship between female weight and oil droplet diameter was statistically weak, however, and future research would benefit from an increased sample size to confirm the results presented here. Maternal age had no effect on any indicators of egg quality. These results highlight the need to understand maternal effects on reproduction in order to evaluate the potential effects of variation in population size structure, and to assess the appropriateness of fishery management strategies for populations that assume all females are reproductively equal.

Maternal influences on egg quality

Maternal length and weight were the main factors that influenced indi-

Global model	Model	df	logLik	AIC_c	ΔAIC_c	w
(a)						
TL	Null	2	106.31	-208.3	0.00	0.39
	<i>HSI</i>	3	106.61	-206.5	1.76	0.16
WE	W	3	59.41	-112.1	0.00	0.29
	$W + HSI + W \times HSI$	5	61.31	-110.7	1.41	0.15
TAG	<i>HSI</i>	3	64.67	-122.6	0.00	0.27
	Null	2	63.05	-121.7	0.88	0.17
	$W + HSI$	4	65.22	-121.2	1.43	0.13
FFA	$A + HSI$	4	64.98	-120.7	1.91	0.10
	Null	2	196.74	-389.1	0.00	0.32
	<i>A</i>	3	197.22	-387.7	1.41	0.16
ST	<i>HSI</i>	3	197.08	-387.4	1.69	0.14
	$W + HSI + W \times HSI$	5	156.54	-301.1	0.00	0.53
PL	<i>W</i>	3	54.68	-102.6	0.00	0.18
	$W + HSI$	4	55.71	-102.2	0.46	0.14
	<i>A</i>	3	54.20	-101.7	0.97	0.11
ED	Null	2	52.86	-101.4	1.26	0.10
	$A + W$	4	55.17	-101.1	1.54	0.08
	$W + HSI + W \times HSI$	5	56.43	-100.9	1.71	0.08
ODD^a	W	3	83.58	-160.4	0.00	0.36
	$W + HSI$	4	84.34	-159.4	1.00	0.22
ODD^a	W	3	139.40	-272.1	0.00	0.26
	$A + W + A \times W$	5	141.39	-270.8	1.23	0.14
	$W + HSI$	4	139.87	-270.5	1.57	0.12
ODD^a	Null	2	137.36	-270.4	1.71	0.11
	(b)					
TL	Null	2	106.31	-208.3	0.00	0.37
	<i>HSI</i>	3	106.61	-206.5	1.76	0.15
WE	FL	3	59.32	-111.9	0.00	0.30
TAG	<i>HSI</i>	3	64.67	-122.4	0.00	0.20
	$FL + HSI$	4	65.87	-122.3	0.13	0.19
	Null	2	63.05	-121.5	0.88	0.13
FFA	$FL + HSI + FL \times HSI$	5	66.54	-121.9	1.48	0.10
	$A + HSI$	4	64.98	-120.7	1.91	0.08
	Null	2	196.74	-389.1	0.00	0.33
ST	<i>A</i>	3	197.22	-387.7	1.41	0.16
	<i>HSI</i>	3	197.08	-387.4	1.69	0.14
	$FL + HSI + FL \times HSI$	5	155.99	-300.0	0.00	0.37
PL	FL	3	152.74	-298.7	1.31	0.19
	<i>FL</i>	3	55.43	-104.1	0.00	0.19
	$FL + HSI$	4	56.55	-103.9	0.28	0.17
ED^a	$FL + A + FL \times A$	5	57.47	-103.0	1.12	0.11
	FL	3	82.52	-158.3	0.00	0.33
	$FL + HSI$	4	82.91	-156.6	1.74	0.14
ODD	Null	2	80.39	-156.4	1.90	0.13
	$FL + A + FL \times A$	5	141.396	-270.9	0.00	0.23
	Null	2	137.357	-270.4	0.50	0.18
ODD	<i>FL</i>	3	138.296	-269.9	0.99	0.14
	<i>A</i>	3	138.224	-269.7	1.13	0.13

^a W was retained as a predictor in the ODD model, and FL was retained as a predictor in the ED model, because these models were significantly better predictors of ODD and ED than their respective null models (chi-squared test, $p < 0.05$)

Table 4. Parameter estimates and overall model fit of selected best model of maternal predictors for each measure of egg quality, including regression coefficient (b) and 95% CI for b . The global model predicting each measure of egg quality was **(a)** weight (W) \times age (A) \times hepatosomatic index (HSI); and **(b)** fork length (FL) \times A \times HSI . Statistically significant parameter estimates: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. TL: total lipid; WE: wax ester; TAG: triacylglycerol; FFA: free fatty acids; ST: sterols; PL: polar lipids; ED : egg diameter; ODD : oil droplet diameter; Egg QI: egg quality indicator

Global model	Predictor	b	t -value	95% CI for b	p
(a)					
TL	(Intercept)	3.07×10^{-2}	13.47	2.62×10^{-2} to 3.52×10^{-2}	***
WE	(Intercept)	3.59×10^{-1}	16.86	3.18×10^{-1} to 4.01×10^{-1}	***
	W	3.73×10^{-5}	2.19	3.86×10^{-6} to 7.08×10^{-5}	*
TAG	(Intercept)	3.43×10^{-1}	46.70	3.28×10^{-1} to 3.57×10^{-1}	***
FFA	(Intercept)	6.11×10^{-4}	3.09	2.23×10^{-4} to 10.00×10^{-4}	**
ST	(Intercept)	7.22×10^{-3}	1.36	-3.19×10^{-3} to 1.76×10^{-2}	>0.05
	W	1.04×10^{-5}	2.29	1.50×10^{-6} to 1.93×10^{-5}	*
	HSI	8.38×10^{-3}	2.54	1.91×10^{-3} to 1.49×10^{-2}	*
	$W \times HSI$	-9.91×10^{-6}	-3.17	-1.60×10^{-5} to -3.78×10^{-6}	**
PL	(Intercept)	2.38×10^{-1}	24.61	2.19×10^{-1} to 2.57×10^{-1}	***
ED	(Intercept)	8.24×10^{-1}	74.29	8.02×10^{-1} to 8.46×10^{-1}	***
	W	2.28×10^{-5}	2.57	5.42×10^{-6} to 4.02×10^{-5}	*
ODD	(Intercept)	1.81×10^{-1}	73.91	1.77×10^{-1} to 1.86×10^{-1}	***
	W	3.97×10^{-6}	2.02	1.18×10^{-7} to 7.82×10^{-6}	0.05
(b)					
TL	(Intercept)	3.07×10^{-2}	13.47	2.62×10^{-2} to 3.52×10^{-2}	***
WE	(Intercept)	2.34×10^{-1}	2.97	7.98×10^{-2} to 3.89×10^{-1}	**
	FL	3.90×10^{-4}	2.14	3.33×10^{-5} to 7.46×10^{-3}	*
TAG	(Intercept)	3.43×10^{-1}	46.70	3.28×10^{-1} to 3.57×10^{-1}	***
FFA	(Intercept)	6.11×10^{-4}	3.09	2.23×10^{-4} to 10.00×10^{-4}	**
ST	(Intercept)	3.35×10^{-2}	5.31	2.11×10^{-2} to 4.59×10^{-2}	***
	FL	-3.94×10^{-5}	-2.70	-6.79×10^{-5} to -1.08×10^{-5}	*
PL	(Intercept)	4.37×10^{-1}	4.99	2.65×10^{-1} to 6.08×10^{-1}	***
	FL	-4.61×10^{-4}	-2.28	-8.57×10^{-4} to -6.51×10^{-5}	*
ED	(Intercept)	7.64×10^{-1}	18.13	6.81×10^{-1} to 8.46×10^{-1}	***
	FL	2.01×10^{-4}	2.07	1.06×10^{-5} to 3.91×10^{-4}	*
ODD	(Intercept)	1.86×10^{-1}	188.8	0.18×10^{-1} to 0.19×10^{-1}	***

cators of egg quality for *P. leopardus*. The positive effect of female size on egg and/or oil droplet size is well quantified in fishes, including hake *Merluccius merluccius* (Mehault et al. 2010) and *Merluccius hubbsi* (Macchi et al. 2013), haddock *Melanogrammus aeglefinus* (Hislop 1988), Atlantic cod *Gadus morhua* (Marteinsdottir & Begg 2002) and stripey sea perch (Evans et al. 2008). This phenotypic effect reflects differing investment in reproductive output by females (Bernardo 1996). The limited influence of body condition (HSI) on indicators of egg quality was, therefore, interesting because body condition is often used to indicate the energetic resources available for reproduction. Female body condition or liver indices have been positively correlated with egg and larval

quality indicators, for example, such as egg and oil droplet size, fertilization success, and larval condition including size at hatching and survival (McCormick 2003, Berkeley et al. 2004a, Trippel & Neil 2004, Donelson et al. 2008, Bachan et al. 2012). The limited effect of body condition on egg quality that we observed may be due to co-variation with another factor not measured.

In batch spawning fishes, egg quality can vary significantly depending on when in the season batches are spawned, with late-season batches often displaying lower quality as the energetic resources available for reproduction diminish (Bachan et al. 2012). An examination of spawning season effects on egg quality indicators was beyond the scope of this study, but a combination of maternal size-related variability and the probable changes in maternal condition during the spawning season for females of a given size, may explain the lack of relationship between condition and egg quality indicators for *P. leopardus*.

Maternal age was not included in any of the models that best described variation in egg or oil droplet size, or egg lipid composition for *P. leopardus*. Maternal age has a positive effect on egg and

Table 5. Percentage change in *Plectropomus leopardus* egg quality indicators with body weight (g, W_{500} to W_{2000}) and fork length (mm, FL_{350} to FL_{500}). Values are included only where the best model was a significantly better predictor than the null model for each egg quality indicator. WE: wax ester; ST: sterols; PL: polar lipids; ED : egg diameter; ODD : oil droplet diameter

	Female metric	
	W_{500} to W_{2000}	FL_{350} to FL_{500}
WE	+16 %	+15 %
ST	-25 %	-30 %
PL	-	-25 %
ED	+4 %	+4 %
ODD	+3 %	-

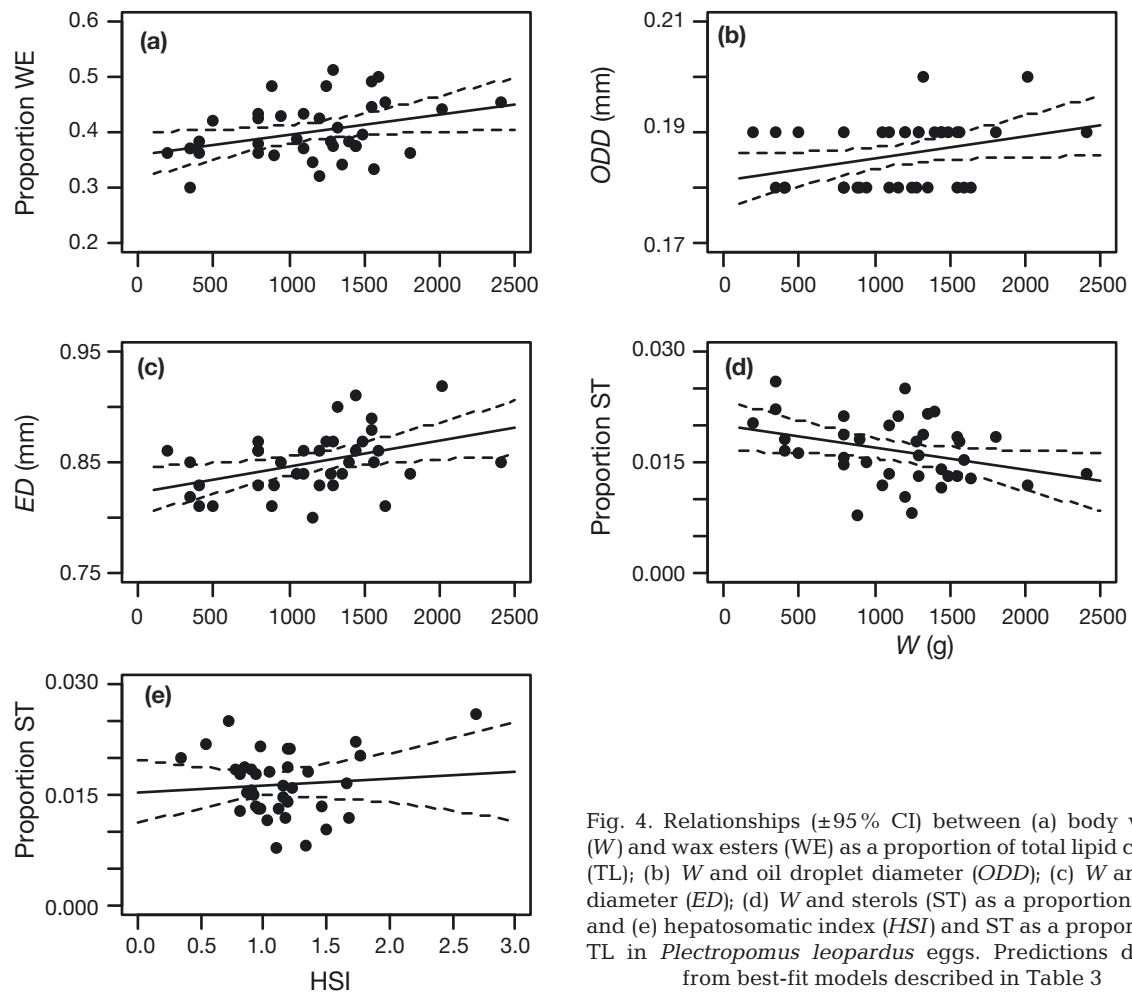


Fig. 4. Relationships ($\pm 95\%$ CI) between (a) body weight (W) and wax esters (WE) as a proportion of total lipid content (TL); (b) W and oil droplet diameter (ODD); (c) W and egg diameter (ED); (d) W and sterols (ST) as a proportion of TL; and (e) hepatosomatic index (HSI) and ST as a proportion of TL in *Plectropomus leopardus* eggs. Predictions derived from best-fit models described in Table 3

larval quality for many temperate fishes, including black rockfish *Sebastes melanops* (Berkeley et al. 2004a), Atlantic cod *G. morhua* (Martensdóttir & Steinarsson 1998) and haddock *M. aeglefinus* (Hislop 1988, Wright & Gibb 2005). Larval *S. melanops* produced by the oldest females, for example, grew 3 times as fast (weight and length) when food was available, and survived for twice as long when food was unavailable, compared to larvae from the youngest females (Berkeley et al. 2004a). Age is rarely the main determinant of egg or larval quality (although see Berkeley et al. 2004a), and usually covaries with maternal size, which is often the better predictor of egg and larval condition (reviewed by Green 2008). The lack of an age effect on egg quality indicators may be indicative of the large amount of variation in female *P. leopardus* age–weight and age–length relationships (Williams et al. 2008), with female size a better indicator of reproductive investment potential than age for this species. Batch fecun-

dity increases with female age for *P. leopardus* (Carter et al. 2014a), indicating that older females invest energy into producing more eggs rather than better quality eggs. However, the positive relationship between age and batch fecundity asymptotes when females are approximately 5 yr old, therefore length and weight remain better indicators of batch fecundity and egg quality than age.

Potential implications for larval quality and recruitment

Polar lipids are the main component of lipoprotein yolk, with some neutral lipids present, while the oil droplet comprises primarily neutral lipids (Wiegand 1996). The yolk sac in *P. leopardus* is utilized during embryogenesis and the first 2 d post hatching. A large portion of the oil droplet remains at 2 d post-hatch when exogenous feeding commences (A. B.

Carter pers. obs.) (Fig. 2c,d), but the oil droplet is not present at 5 d post-hatching (Masuma et al. 1993). This pattern is consistent with many marine larvae where the lipoprotein yolk provides the principal source of energy during embryogenesis, and the oil droplet is consumed post-hatching (Anderson et al. 1990, Rønnestad et al. 1998). The compartmentalization of neutral lipids between lipoprotein yolk and oil is most likely for nutritional reasons, where the oil droplet is retained as an energy reserve in the event that food is limited in the early stages of exogenous feeding, and the oil is consumed to enhance growth when adequate food is available (Wiegand 1996). These endogenous energy reserves are important for larval survival because larval mortality during the transition from endogenous to exogenous feeding is frequently high (Moodie et al. 1989), and thought to be a significant source of recruitment fluctuations (Lasker 1981).

Egg and oil droplet size, and the polar and neutral lipid classes we measured, theoretically are good predictors of embryonic development and larval growth and survival. For many fishes, the oil droplet contains the majority of egg energy content, in particular the energy required to sustain larvae during development and the transition to exogenous feeding (Wiegand 1996, Rønnestad et al. 1998). Neutral lipids within the oil droplet provide endogenous energy for metabolism and growth, with WE and TAG the dominant neutral lipids in fish eggs with an oil droplet (Wiegand 1996). In *P. leopardus* eggs, WE and TAG accounted for approximately 74% of TL. The dominance of neutral lipids relative to PL is typical of warmer water species (Mourente & Odriozola 1990, Navas et al. 1997), while PL are generally the dominant lipid class in cold water fish eggs (Tocher & Sargent 1984). TAG lipids are a short-term energy storage lipid and constitute the major resource available to developing embryos (Hilton et al. 2008). They are also the primary reserve lipid mobilized by larvae during starvation (Rainuzzo et al. 1997). The lack of maternal effects on TAG composition in *P. leopardus* eggs indicates that the provisioning of TAG is tightly regulated in this species irrespective of female size, age or condition. Maternal size did, however, have a positive effect on the provision of the long-term energy storage lipid WE in *P. leopardus* eggs. The benefits of greater WE content in *P. leopardus* eggs produced by larger females is unclear, but higher levels are likely to be advantageous for larvae due to the potential role of WE in energy reserves and buoyancy. Previous studies on plankton have demonstrated that TAG lipids are the primary initial energy

for larvae, but once these are depleted the starvation-related stress activates the wax-lipase and WE are consumed (Lee et al. 1974). An increased portion of WE in the egg may, therefore, provide larvae that are spawned from larger females with a potential survival advantage during the transition to exogenous feeding.

PL are important during embryogenesis as they provide energy reserves and are an important structural element in biomembrane formation (Wiegand 1996). For fishes that produce eggs with an oil droplet, the proportion of PL can range widely, e.g. 6% of TL in golden perch eggs (Anderson et al. 1990) and 72% of TL in striped trumpeter eggs (Bransden et al. 2007). In *P. leopardus* eggs, approximately 24% of TL content was PL. The decrease in PL with female length does not necessarily indicate that larger *P. leopardus* produce eggs with lower quality yolk than eggs produced by smaller females. Decreasing PL concentrations in eggs spawned by larger females would be compensated to some extent by the increase in egg size and, therefore, an increase in the amount of total PL available for embryogenesis. Furthermore, the higher PL concentrations in eggs spawned by the smallest *P. leopardus* also generally had reduced WE and TAG concentrations and low TL content (Table 2), with potentially negative consequences for larval survival. For example, increases in PL and decreases in TAG have been reported over the spawning season in the striped trumpeter (Bransden et al. 2007), where the shift in lipid composition was indicative of the typical decrease in egg quality experienced by batch spawning fishes as maternal resources diminish over time (Bachan et al. 2012).

Maternal reproductive success is a product of reproductive potential and offspring survival (Lambert 2008). The ability of large *P. leopardus* to produce relatively large eggs with a large oil droplet, with a constant TAG concentration and increased WE concentration, likely has important implications for larval quality and survival. Larvae with high energy reserves are less susceptible to starvation during the period between endogenous and exogenous feeding (Moodie et al. 1989, Wiegand 1996, Brooks et al. 1997). The large number of studies that have examined the effect of egg and/or oil droplet size on larval quality and reproductive success have shown that larger eggs generally result in greater fertilization rates (e.g. common snook *Centropomus undecimalis* [Neidig et al. 2000]), greater larval size at hatching that persists through time (e.g. Atlantic cod *G. morhua* [Marteinsdottir & Begg 2002] and brown trout *Salmo trutta* [Einum & Fleming 1999]),

faster rates of larval growth (e.g. walleye *Stizostedion vitreum* [Moodie et al. 1989]), faster larval development and survival rates (e.g. black rockfish *S. melanops* [Berkeley et al. 2004a]), and greater larval feeding success (e.g. Atlantic cod *G. morhua* [Knutsen & Tilseth 1985]). In terms of egg biochemistry, lipids are good indicators of egg quality due to their importance, particularly of neutral lipids, in embryogenesis, hatching success, larval growth and larval survival (Wiegand 1996, Navas et al. 1997, Berkeley et al. 2004a, Hilton et al. 2008). Increased buoyancy of larger eggs and/or eggs with a greater proportion of WE may also have positive implications for the survival of larval *P. leopardus*, as buoyancy positions eggs and newly hatched larvae in the upper layers of the water column where larval food sources are most abundant and oxygen conditions are best (Wright & Fyhn 2001, Mehault et al. 2010). Larger larvae with faster growth rates potentially have a survival advantage as they are able to outgrow smaller predators faster and therefore experience relatively less predation than smaller conspecifics (Vigliola & Meekan 2002), are able to access more of the water column in a retention area than smaller conspecifics due to increased swimming ability (Álvarez-Colombo et al. 2011), and can start feeding earlier and have higher feeding success as they are able to eat a wider variety of food items (Knutsen & Tilseth 1985).

Species-specific assessments of a wide range of indicators of egg and larval quality are required, as the relative importance of egg quality indicators is likely to be closely related to the early life-history requirements of that species (Kaitaranta & Ackman 1981). Care should be taken in assuming the theory that egg size or biochemical composition will relate to embryo or larval performance in all species because there are many exceptions. For example, there is no relationship between egg morphometry and fertilization and hatching rates for Asian sea bass *Lates calcarifer* (Nocillado et al. 2000), or between egg size and embryo and larval development for the winter flounder *Pseudopleuronectes americanus* (Butts & Litvak 2007), and no difference in egg PL, TAG and TL composition between viable and unviable eggs for Atlantic halibut *Hippoglossus hippoglossus* (Bruce et al. 1993). In addition, egg quality can vary within the spawning season for batch spawners (Bachan et al. 2012), and vary depending on maternal stress caused by social conditions such as crowding (McCormick 2006). Paternal effects may also influence the quality of fertilized eggs and subsequent larvae, including larval size, growth and performance (Brooks et al.

1997, Green & McCormick 2005, Butts & Litvak 2007). Further research is required to determine the effect that variation in egg morphometry and lipid composition has on fertilization success, hatching rates and larval survival for *P. leopardus*, and on other potentially important sources of variation in egg quality.

Recruitment success is positively influenced by larval growth and survival (Bergenius et al. 2002, Fontes et al. 2011). Variation in the egg quality traits that influence larval growth and survival for *P. leopardus*, particularly during the critical period between hatching and the establishment of exogenous feeding, may have significant implications for recruitment of this species. Fisheries models generally assume that eggs and larvae produced by females are of equal quality, regardless of the size and age structure of those populations (Birkeland & Dayton 2005). Our study demonstrates that large female *P. leopardus* produce higher quality eggs than those spawned by smaller females. Batch fecundity also increases with female length and weight for *P. leopardus* (Carter et al. 2014a), as does the proportion of females that are reproductively mature (Carter et al. 2014b). Protection of large females is afforded in 33% of the GBR Marine Park (GBRMP), which is closed to fishing (no-take marine reserves), where the mean length at female–male sex change, mean length and weight of female spawners, weight-specific batch fecundity (central GBR), and overall densities of coral trout, are greater relative to fished reefs (Carter et al. 2014a,b, Emslie et al. 2015). The combination of direct removal of large females through fishing, and indirect removal of large females via fishing-induced female–male sex change at smaller sizes relative to reserve reefs, is likely to have deleterious consequences for the quality of eggs produced by *P. leopardus* populations on reefs open to fishing. The ‘indirect’ removal of females is of particular concern in tropical fisheries management where protogynous hermaphroditism is prevalent (Jennings & Kaiser 1998) and exploited species such as *P. leopardus* are likely to be particularly susceptible to fishing-induced reductions in egg quality. No-take reserves in the GBRMP are, therefore, likely to have positive effects on the quality and quantity of *P. leopardus* eggs produced, which are likely to supplement populations of *P. leopardus* on fished reefs (Harrison et al. 2012).

This is the first study to examine the effects of maternal length, weight, age and body condition on egg size and lipid composition for a commercially important tropical fish of wild origin. This study is an

important addition to the growing body of literature on the importance of large female fish for population reproductive output in terms of the quality of eggs produced. Our findings support the hypothesis that larger female fish produce better quality eggs, but not the hypothesis that older females produce better quality eggs. The reproductive importance of large females has notable implications for the management of coral trout fisheries. Through the selective removal of the largest individuals, fishing takes away, or in the very least diminishes, the reproductive benefits that larger females can provide for population viability. The effect of female size on egg quality and the potential implications for early survival and growth of larvae and recruitment is particularly relevant to exploited species like *P. leopardus*, from which fishing removes larger and older females of the population.

Acknowledgements. The authors thank Elio Parenti for assisting with sample collection, and Kylie and Rob Dodds for assistance on Orpheus Island Research Station. Thanks to Ann Penny, Leanne Currey, Shane Brown, Harrison Carter-Brown, Margaret Carter, Kara Dew and Renae Tobin for assistance with field work and/or sample processing. Lipid extraction and analysis was conducted at CSIRO Laboratories, Hobart, Tasmania, with particular thanks to Heidi Pethybridge. Otolith processing and age estimates were conducted by Dong C. Lou. Rhondda Jones and Susan Jacups (James Cook University) provided valuable statistical advice. Partial funding for this project was provided by Great Barrier Reef Marine Park Authority Science for Management Awards and a PADI Project Aware grant awarded to A.B.C. A.B.C. was supported by a James Cook University Postgraduate Research Scholarship. This research was conducted under ethics and research permits granted to A.B.C. and A.G.C. by James Cook University, the Great Barrier Reef Marine Park Authority and Queensland's Department of Primary Industries and Fisheries (now Department of Agriculture and Fisheries). Comments from 3 anonymous reviewers greatly improved the manuscript.

LITERATURE CITED

- Álvarez-Colombo G, Dato C, Macchi G, Palma E and others (2011) Distribution and behavior of Argentine hake larvae: Evidence of a biophysical mechanism for self-recruitment in northern Patagonian shelf waters. *Cienc Mar* 37:633–657
- Anderson A, Arthington A, Anderson S (1990) Lipid classes and fatty acid composition of the eggs of some Australian fish. *Comp Biochem Physiol B* 96:267–270
- Bachan MM, Fleming IA, Trippel EA (2012) Maternal allocation of lipid classes and fatty acids with seasonal egg production in Atlantic cod (*Gadus morhua*) of wild origin. *Mar Biol* 159:2281–2297
- Barton K (2013) MuMIn: multi-model inference. R package Version 1.9.13. Available at <http://CRAN.R-project.org/package=MuMIn> (accessed on 4 June 2013)
- Bates D, Maechler M, Bolker B (2012) lme4: linear mixed-effects models using Eigen and S4. R package Version 0.999999-0. Available at <http://CRAN.R-project.org/package=lme4> (accessed on 4 June 2013)
- Bergenius MA, Meekan MG, Robertson RD, McCormick MI (2002) Larval growth predicts the recruitment success of a coral reef fish. *Oecologia* 131:521–525
- Berkeley SA, Chapman C, Sogard SM (2004a) Maternal age as a determinant of larval growth and survival in a marine fish, *Sebastes melanops*. *Ecology* 85:1258–1264
- Berkeley SA, Hixon MA, Larson RJ, Love MS (2004b) Fisheries sustainability via protection of age structure and spatial distribution of fish populations. *Fisheries* 29: 23–32
- Bernardo J (1996) The particular maternal effect of propagule size, especially egg size: patterns, models, quality of evidence and interpretations. *Am Zool* 36:216–236
- Birkeland C, Dayton PK (2005) The importance in fishery management of leaving the big ones. *Trends Ecol Evol* 20:356–358
- Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* 37: 911–917
- Brandsen M, Battaglione S, Goldsmid R, Dunstan G, Nichols P (2007) Broodstock condition, egg morphology and lipid content and composition during the spawning season of captive striped trumpeter, *Latris lineata*. *Aquaculture* 268:2–12
- Brooks S, Tyler CR, Sumpter JP (1997) Egg quality in fish: What makes a good egg? *Rev Fish Biol Fish* 7:387–416
- Bruce M, Shields R, Bell M, Bromage N (1993) Lipid class and fatty acid composition of eggs of Atlantic halibut, *Hippoglossus hippoglossus* (L.), in relation to egg quality in captive broodstock. *Aquac Res* 24:417–422
- Burnham KP, Anderson DR (2002) Model selection and multi-model inference: a practical information-theoretic approach, 2nd edn. Springer, New York, NY
- Butts IA, Litvak MK (2007) Parental and stock effects on larval growth and survival to metamorphosis in winter flounder (*Pseudopleuronectes americanus*). *Aquaculture* 269:339–348
- Carter AB, Davies CR, Mapstone BD, Russ GR, Tobin AJ, Williams AJ (2014a) Effects of region, demography, and protection from fishing on batch fecundity of common coral trout (*Plectropomus leopardus*). *Coral Reefs* 33: 751–763
- Carter AB, Davies CR, Mapstone BD, Russ GR, Tobin AJ, Williams AJ (2014b) Spatial variation in the effects of size and age on reproductive dynamics of common coral trout *Plectropomus leopardus*. *J Fish Biol* 84:1074–1098
- Claramunt G, Serra R, Castro LR, Cubillos L (2007) Is the spawning frequency dependent on female size? Empirical evidence in *Sardinops sagax* and *Engraulis ringens* off northern Chile. *Fish Res* 85:248–257
- Cornish A, Kiwi LK (2004) *Plectropomus leopardus*. IUCN Red List of Threatened Species Version 2013.2. Available at www.iucnredlist.org/apps/redlist/details/44684/0 (accessed on 24 February 2014)
- Donelson JM, McCormick MI, Munday PL (2008) Parental condition affects early life-history of a coral reef fish. *J Exp Mar Biol Ecol* 360:109–116
- Einum S, Fleming IA (1999) Maternal effects of egg size in brown trout (*Salmo trutta*): norms of reaction to environmental quality. *Proc R Soc Lond B* 266:2095–2100
- Emslie MJ, Logan M, Williamson DH, Ayling AM and others

- (2015) Expectations and outcomes of reserve network performance following re-zoning of the Great Barrier Reef Marine Park. *Curr Biol* 25:983–992
- Evans RD, Russ GR, Kritzer JP (2008) Batch fecundity of *Lutjanus carponotatus* (Lutjanidae) and implications of no-take marine reserves on the Great Barrier Reef, Australia. *Coral Reefs* 27:179–189
- Ferreira BP, Russ GR (1994) Age validation and estimation of growth rate of the coral trout, *Plectropomus leopardus*, (Lacepede 1802) from Lizard Island, Northern Great Barrier Reef. *Fish Bull* 92:46–57
- Fontes J, Santos RS, Afonso P, Caselle JE (2011) Larval growth, size, stage duration and recruitment success of a temperate reef fish. *J Sea Res* 65:1–7
- Fox J, Weisberg S (2009) Car: companion to applied regression. R package Version 02-14. Available at <http://cran.r-project.org/web/packages/car> (accessed on 24 February 2014)
- Green BS (2008) Maternal effects in fish populations. *Adv Mar Biol* 54:1–105
- Green BS, McCormick MI (2005) Maternal and paternal effects determine size, growth and performance in larvae of a tropical reef fish. *Mar Ecol Prog Ser* 289:263–272
- Harrison HB, Williamson DH, Evans RD, Almany GR and others (2012) Larval export from marine reserves and the recruitment benefit for fish and fisheries. *Curr Biol* 22:1023–1028
- Hawkins JP, Roberts CM (2004) Effects of fishing on sex-changing Caribbean parrotfishes. *Biol Conserv* 115:213–226
- Heemstra PC, Randall JE (1993) FAO species catalogue Vol 16: Groupers of the world (family Serranidae, subfamily Epinephelinae): An annotated and illustrated catalogue of the grouper, rockcod, hind, coral grouper and lyretail species known to date. FAO Fisheries Synopsis No 125, Food and Agriculture Organization of the United Nations, Rome
- Hilton Z, Poortenaar CW, Sewell MA (2008) Lipid and protein utilisation during early development of yellowtail kingfish (*Seriola lalandi*). *Mar Biol* 154:855–865
- Hislop J (1988) The influence of maternal length and age on the size and weight of the eggs and the relative fecundity of the haddock, *Melanogrammus aeglefinus*, in British waters. *J Fish Biol* 32:923–930
- Hixon MA, Johnson DW, Sogard SM (2014) BOFFFFs: on the importance of conserving old-growth age structure in fishery populations. *ICES J Mar Sci* 71:2171–2185
- Hutchings JA (1991) Fitness consequences of variation in egg size and food abundance in brook trout *Salvelinus fontinalis*. *Evolution* 45:1162–1168
- Jennings S, Kaiser MJ (1998) The effects of fishing on marine ecosystems. *Adv Mar Biol* 34:201–352
- Kaitaranta JK, Ackman RG (1981) Total lipids and lipid classes of fish roe. *Comp Biochem Physiol B* 69:725–729
- Kamler E (2005) Parent–egg–progeny relationships in teleost fishes: an energetics perspective. *Rev Fish Biol Fish* 15:399–421
- Kjørsvik E (1994) Egg quality in wild and broodstock cod *Gadus morhua* L. *J World Aquac Soc* 25:22–29
- Kjørsvik E, Mangor-Jensen A, Holmeford I (1990) Egg quality in fishes. *Adv Mar Biol* 26:71–113
- Knutsen G, Tilseth S (1985) Growth, development, and feeding success of Atlantic cod larvae *Gadus morhua* related to egg size. *Trans Am Fish Soc* 114:507–511
- Lambert Y (2008) Why should we closely monitor fecundity in marine fish populations. *J Northwest Atl Fish Sci* 41:93–106
- Lasker R (1981) The role of a stable ocean in larval fish survival and subsequent recruitment. University of Washington Press, Seattle, WA
- Lee RF, Nevenzel JC, Lewis A (1974) Lipid changes during life cycle of marine copepod, *Euchaeta japonica* Marukawa. *Lipids* 9:891–898
- Lowerre-Barbieri SK, Henderson N, Llopiz J, Walters S, Bickford J, Muller R (2009) Defining a spawning population (spotted seatrout *Cynoscion nebulosus*) over temporal, spatial, and demographic scales. *Mar Ecol Prog Ser* 394:231–245
- Macchi GJ, Leonarduzzi E, Diaz MV, Renzi M, Rodrigues K (2013) Maternal effects on fecundity and egg quality of the Patagonian stock of Argentine hake (*Merluccius hubbsi*). *Fish Bull* 111:325–336
- Maddams JC, McCormick MI (2012) Not all offspring are created equal: Variation in larval characteristics in a serially spawning damselfish. *PLoS ONE* 7:e48525
- Mapstone B, Little L, Punt A, Davies C and others (2008) Management strategy evaluation for line fishing in the Great Barrier Reef: Balancing conservation and multi-sector fishery objectives. *Fish Res* 94:315–329
- Marteinsdóttir G, Begg GA (2002) Essential relationships incorporating the influence of age, size and condition on variables required for estimation of reproductive potential in Atlantic cod *Gadus morhua*. *Mar Ecol Prog Ser* 235:235–256
- Marteinsdóttir G, Steinarsson A (1998) Maternal influence on the size and viability of Iceland cod *Gadus morhua* eggs and larvae. *J Fish Biol* 52:1241–1258
- Masuma S, Tezuka N, Teruya K (1993) Embryonic and morphological development of larval and juvenile coral trout, *Plectropomus leopardus*. *Jpn J Ichthyol* 40:333–342
- McCormick MI (2003) Consumption of coral propagules after mass spawning enhances larval quality of damselfish through maternal effects. *Oecologia* 136:37–45
- McCormick MI (2006) Mothers matter: Crowding leads to stressed mothers and smaller offspring in marine fish. *Ecology* 87:1104–1109
- McCormick M, Gagliano M (2008) Carry-over effects—the importance of a good start. *Proc 11th Int Coral Reef Symp, Fort Lauderdale, FL*, p 305–310
- Mehault S, Domínguez-Petit R, Cerviño S, Saborido-Rey F (2010) Variability in total egg production and implications for management of the southern stock of European hake. *Fish Res* 104:111–122
- Migaud H, Bell G, Cabrita E, McAndrew B and others (2013) Gamete quality and broodstock management in temperate fish. *Rev Aquac* 5:S194–S223
- Moodie G, Loadman N, Wiegand M, Mathias J (1989) Influence of egg characteristics on survival, growth and feeding in larval walleye (*Stizostedion vitreum*). *Can J Fish Aquat Sci* 46:516–521
- Mourente G, Odriozola J (1990) Effect of broodstock diets on lipid classes and their fatty acid composition in eggs of gilthead sea bream (*Sparus aurata* L.). *Fish Physiol Biochem* 8:93–101
- Navas J, Bruce M, Thrush M, Farndale B and others (1997) The impact of seasonal alteration in the lipid composition of broodstock diets on egg quality in the European sea bass. *J Fish Biol* 51:760–773
- Neidig CL, Skapura DP, Grier HJ, Dennis CW (2000) Tech-

- niques for spawning common snook: broodstock handling, oocyte staging, and egg quality. *N Am J Aquac* 62: 103–113
- Nichols PD, Nichols DS, Bakes MJ (1994) Developments with Marine Oil Products in Australia. *Int News Fats Oils Relat Mater* 5:254–261
- Nocillado J, Penaflores V, Borlongan I (2000) Measures of egg quality in induced spawns of the Asian sea bass, *Lates calcarifer* Bloch. *Fish Physiol Biochem* 22:1–9
- R Development Core Team (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
- Rainuzzo JR, Reitan KI, Olsen Y (1997) The significance of lipids at early stages of marine fish: a review. *Aquaculture* 155:103–115
- Rønnestad I, Koven W, Tandler A, Harel M, Fyhn HJ (1998) Utilisation of yolk fuels in developing eggs and larvae of European sea bass (*Dicentrarchus labrax*). *Aquaculture* 162:157–170
- Sadovy YJ (1996) Reproduction of reef fishery species. In: Polunin NVC, Roberts CM (eds) Reef fisheries. Chapman and Hall, London, p 15–59
- Sadovy de Mitcheson Y, Craig MT, Bertoncini AA, Carpenter KE and others (2013) Fishing groupers towards extinction: a global assessment of threats and extinction risks in a billion dollar fishery. *Fish Fish* 14:119–136
- Samoilys MA (2000) Reproductive dynamics of an exploited serranid on the Great Barrier Reef. PhD thesis, James Cook University, Townsville
- Tocher DR (2003) Metabolism and functions of lipids and fatty acids in teleost fish. *Rev Fish Sci* 11:107–184
- Tocher DR, Sargent JR (1984) Analyses of lipids and fatty acids in ripe roes of some northwest European marine fish. *Lipids* 19:492–499
- Trippel EA, Neil SRE (2004) Maternal and seasonal differences in egg sizes and spawning activity of northwest Atlantic haddock (*Melanogrammus aeglefinus*) in relation to body size and condition. *Can J Fish Aquat Sci* 61: 2097–2110
- Venturelli PA, Shuter BJ, Murphy CA (2009) Evidence for harvest-induced maternal influences on the reproductive rates of fish populations. *Proc R Soc Lond B* 276:919–924
- Vigliola L, Meekan MG (2002) Size at hatching and planktonic growth determine post-settlement survivorship of a coral reef fish. *Oecologia* 131:89–93
- Wiegand MD (1996) Composition, accumulation and utilization of yolk lipids in teleost fish. *Rev Fish Biol Fish* 6: 259–286
- Williams AJ, Currey LM, Begg GA, Murchie CD, Ballagh AC (2008) Population biology of coral trout species in eastern Torres Strait: implications for fishery management. *Cont Shelf Res* 28:2129–2142
- Wootton R (1977) Effect of food limitation during the breeding season on the size, body components and egg production of female sticklebacks (*Gasterosteus aculeatus*). *J Anim Ecol* 46:823–834
- Wright P, Fyhn H (2001) Ontogeny of nitrogen metabolism and excretion. *Fish Physiol* 20:149–200
- Wright PJ, Gibb FM (2005) Selection for birth date in North Sea haddock and its relation to maternal age. *J Anim Ecol* 74:303–312
- Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM (2009) Mixed effects models and extensions in ecology with R. Springer, New York, NY

Editorial responsibility: Stylianos Somarakis, Heraklion, Greece

Submitted: July 2, 2014; Accepted: March 12, 2015
Proofs received from author(s): May 20, 2015