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RESEARCH ARTICLE

A multilevel approach to examining cephalopod growth using *Octopus pallidus* as a model

Jayson Semmens[1,*], Zoë Doubleday[1], Kate Hoyle[2,†] and Gretta Pecl[1]

[1]Fisheries, Aquaculture and Coasts Centre, Institute for Marine and Antarctic Studies, University of Tasmania, Private Bag 49, Tasmania 7001, Australia and [2]School of Zoology, University of Tasmania, Private Bag 5, Tasmania 7001, Australia

*Author for correspondence (jayson.semmens@utas.edu.au)

†Present address: Water Assessment Branch, Department of Primary Industries, Parks, Water and Environment, 13 St Johns Avenue, New Town, Tasmania 7008, Australia

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SUMMARY

Many aspects of octopus growth dynamics are poorly understood, particularly in relation to sub-adult or adult growth, muscle fibre dynamics and repro-somatic investment. The growth of 5 month old *Octopus pallidus* cultured in the laboratory was investigated under three temperature regimes over a 12 week period: seasonally increasing temperatures (14–18°C); seasonally decreasing temperatures (18–14°C); and a constant temperature mid-way between seasonal peaks (16°C). Differences in somatic growth at the whole-animal level, muscle tissue structure and rate of gonad development were investigated. Continuous exponential growth was observed, both at a group and at an individual level, and there was no detectable effect of temperature on whole-animal growth rate. Juvenile growth rate (from 1 to 156 days) was also monitored prior to the controlled experiment; exponential growth was observed, but at a significantly faster rate than in the older experimental animals, suggesting that *O. pallidus* exhibit a double-exponential two-phase growth pattern. There was considerable variability in size-at-age even between individuals growing under identical thermal regimes. Animals exposed to seasonally decreasing temperatures exhibited a higher rate of gonad development compared with animals exposed to increasing temperatures; however, this did not coincide with a detectable decline in somatic growth rate or mantle condition. The ongoing production of new mitochondria-poor and mitochondria-rich muscle fibres (hyperplasia) was observed, indicated by a decreased or stable mean muscle fibre diameter concurrent with an increase in whole-body size. Animals from both seasonal temperature regimes demonstrated higher rates of new mitochondria-rich fibre generation relative to those from the constant temperature regime, but this difference was not reflected in a difference in growth rate at the whole-body level. This is the first study to record ongoing hyperplasia in the muscle tissue of an octopus species, and provides further insight into the complex growth dynamics of octopus.

Key words: growth, muscle fibre dynamics, repro-somatic investment, seasonal temperature regime, cephalopod, octopus.

INTRODUCTION

Cephalopods typically exhibit rapid and non-asymptotic growth over short life spans, which usually range between 6 months and 2 years (Forsythe and Van Heukelem, 1987; Jackson and O'Dor, 2001; Leporati et al., 2008b; Moltschaniwskyj, 2004). The general form of cephalopod growth has been best described by exponential and power curves, although linear and logarithmic curves have also been used (Alford and Jackson, 1993; Semmens et al., 2004). Cephalopods are also capable of dramatic growth plasticity: at an inter-specific level, at a population level and even at an individual level. Growth rates may be highly variable, or variability may be evident in differences in size-at-age and size-at-maturity (e.g. Leporati et al., 2008a; Leporati et al., 2008b). Because of the nature of exponential or non-asymptotic growth, small inherent individual variations in growth rates or initial size at hatching may be magnified over time to produce large differences in size-at-age within a population (André et al., 2009b). Laboratory culture studies have consistently suggested that cephalopod growth occurs in two phases over the life cycle (e.g. DeRusha et al., 1987; Forsythe and Hanlon, 1988; Segawa and Nomoto, 2002), which typically consists of an early phase of rapid exponential growth followed by a slower phase, best described by a power equation (Semmens et al., 2004); however, there is considerable debate about whether two-phase growth occurs in the wild (André et al., 2009b). These unique growth characteristics have important implications for a species' population dynamics and productivity and how they respond to environmental change and variability. Furthering our understanding of these growth characteristics, therefore, is vital for the effective management, conservation and aquaculture development of cephalopod species.

The mechanism allowing continuous growth in cephalopods is related to the growth processes of the muscle, which makes up about 90% of the body mass (Moltschaniwskyj, 1994). Muscle growth involves two processes: (i) hyperplasia, the generation of new muscle fibres; and (ii) hypertrophy, the increase in size of those fibres already in existence (Rowlerson et al., 1995; Weatherley, 1990). If growth is asymptotic, as with the vast majority of teleosts, hyperplasia ceases at some point in the life cycle (Weatherley and Gill, 1987). Further growth can occur only by hypertrophy of the existing fibres, which will also eventually cease because of limitations of fibre size. In teleosts, the slowing of growth, due to the physiological limitations of fibre size, allows energy previously used for somatic growth to be stored or used for reproductive growth. Non-asymptotic growth, therefore, has implications for the level of

repro-somatic investment that may or may not be achievable for an individual (Moltschaniwskyj, 2004). To date, ongoing hyperplasia has been reported for four species of squid [*Sepioteuthis australis* (Ho et al., 2004); *Nototodarus gouldi* (McGrath Steer, 2003); *Photololigo* sp. (Moltschaniwskyj, 1994); *Loligo opalescens* (Preuss et al., 1997)], one species of cuttlefish [*Sepia elliptica* (Martínez and Moltschaniwskyj, 1999)] and one species of sepiolid [*Idiosepius pygmaeus* (Pecl and Moltschaniwskyj, 1997)]. The ongoing production of new, small fibres means that these species have the capacity for continuous somatic growth without being limited by the physiological constraints of maximum muscle fibre size. Additionally, the metabolic advantages of small cell size are maximised by sustained hyperplasia, thereby facilitating the rapid growth rates achieved by cephalopods. While high growth rates and non-asymptotic growth curves have been reported for octopus [e.g. *Octopus joubini* (Forsythe, 1984); *Octopus burryi* (Forsythe and Hanlon, 1985); *Octopus bimaculoides* (Forsythe and Hanlon, 1988); *Octopus pallidus* (Leporati et al., 2007)], sustained hyperplasia is yet to be observed in any octopus species.

Cephalopod growth processes are responsive to a variety of abiotic factors, with temperature being the main factor influencing growth rate and growth variability if food is not limiting (Forsythe and Van Heukelem, 1987; Forsythe et al., 2001; Leporati et al., 2007; Villanueva, 2000). The juvenile growth phase is considered to be particularly sensitive to temperature (Semmens et al., 2004). For example, Forsythe conducted simulations based on laboratory growth rates of the squid *Loligo forbesi* and found that even a 1°C increase in average temperature over the 90 day exponential growth period produced individuals double the size of their counterparts (Forsythe, 1993). This experiment formed the basis of the 'Forsythe effect' (Forsythe, 2004; Forsythe, 1993), a key hypothesis that explains how growth in cephalopods is influenced by temperature. The hypothesis states that as hatching occurs over a period of increasing water temperatures, each new 'cohort' will be exposed to higher temperatures, and thus will grow significantly faster than those that hatched only weeks previously. Growth rates of juvenile octopus in captivity have also been found to be highly influenced by temperature (e.g. Forsythe and Hanlon, 1988; Leporati et al., 2007; Segawa and Nomoto, 2002), and modelled projections of octopus growth also suggest that increases of only 1°C can affect body mass by up to 62.6% in 100 days (André et al., 2009a). Laboratory studies indicate that temperature does not affect the sub-adult or adult growth phase in cephalopods in any consistent way; however, a shortcoming of these studies is the routine use of artificial constant temperature regimes, which are limiting our understanding of exactly how temperature affects cephalopod growth in nature (Forsythe, 1993; Forsythe and Hanlon, 1988). To date, only one known study has utilised simulated seasonal temperature regimes to examine octopus growth in the laboratory, with Leporati and colleagues examining the growth of juveniles under such regimes (Leporati et al., 2007), which provided a clearer understanding of how juvenile octopus respond to increasing and decreasing temperature regimes; therefore, more such studies, which better simulate natural conditions and therefore natural growth patterns, are required to examine adult octopus growth more realistically.

The overall objective of this study was to investigate the growth dynamics of *Octopus pallidus*, Hoyle 1885, at three different levels, and assess the way in which growth is affected by temperature using simulated seasonal temperature regimes. *Octopus pallidus* is a holobenthic (i.e. produces large well-developed benthic young) terminal spawner and is commonly found in temperate south east Australian waters (Stranks, 1996). *Octopus pallidus* forms the basis of a commercial fishery in Bass Strait, Tasmania, Australia, with an annual catch of approximately 80 tonnes. There has been considerable recent research conducted on *O. pallidus* (e.g. André et al., 2008; Doubleday et al., 2008; Leporati et al., 2008b), and the species serves as a good model to examine detailed aspects of octopus growth in the laboratory. The key aims of our study were to determine: (1) the nature of the growth curve from hatching to 8 months old, (2) the mechanism of muscle growth (hyperplasia *versus* hypertrophy), and (3) the effect of seasonal temperature on whole-body growth, muscle tissue structure and repro-somatic investment, with the hypothesis that females will be ready to spawn during cooler periods, such that hatching young will be exposed to continually increasing temperatures for the first few months of life when growth is rapid (Forsythe, 1993).

MATERIALS AND METHODS

Growth of *O. pallidus* under three different temperature regimes was investigated in a fully replicated laboratory experiment of 100 days duration. Although it would have been preferable to conduct the experiment over the entire lifetime of the species, it was not possible at the time because of resource constraints, and the examination of sub-adult and adult growth was the key aim of the study. The experiment used 72 individuals reared in captivity from a single egg batch laid in a PVC sediment-collector in Wedge Bay, Tasmania, Australia (43°06′S, 147°43′E) in August 2001. The newly laid eggs and the brooding female were transferred to a 500 l aquaria connected to a flow-through seawater system and exposed to ambient temperatures (ranging between 8.4 and 16.3°C) over a development period of 121 days (1 August 2001 to 29 November 2001). On hatching, 179 animals were transferred to three 500 l aquarium and maintained for 156 days, prior to transfer to experimental tanks. Octopuses were exposed to ambient temperatures over this period (ranging between 14 and 17.9°C; mean: 16.1±0.07°C) and fed *ad libitum* on live crabs (*Petrolisthes elongatus*). To examine juvenile growth prior to the manipulative experiment, a random sub-sample of animals were weighed at days 1 (hatching), 9, 20, 32, 84 and 156 (total N=535).

The experiment comprised three temperature treatments: simulated seasonal increasing temperature (14–18°C over 12 weeks); simulated seasonal decreasing temperature (18–14°C over 12 weeks); and a constant temperature (16°C for 12 weeks). The increasing and decreasing temperature regimes were based on Tasmanian inshore water temperatures recorded over 3 months in late spring/early summer and late autumn/early winter, respectively. Six circular 30 l tanks were allocated to each treatment, each containing four animals separated by mesh screens (n=24 per treatment, N=72). Tanks were connected to a flow-through natural seawater system, and arranged in a block design of three rows, each row corresponding to a particular treatment. All tanks were enclosed within a black plastic partition, and exposed equally to a 12 h L:12 h D photoperiod using artificial lighting and equal levels of disturbance. For the duration of the experiment animals were fed *ad libitum* on a diet of live crabs (*P. elongatus*). Each individual was provided with a length of PVC pipe as a refuge and all tanks were cleaned and maintained fortnightly. Prior to commencement of the experiment, animals were transferred to the experimental tanks at ambient temperature and acclimated at a rate ≤1°C per day until all tanks had reached the designated starting temperatures (decreasing, 18°C; constant, 16°C; increasing, 14°C). No signs of stress (i.e. inking, swimming erratically and rapid colour change) were observed during the acclimatisation period. Following the start date, temperatures were raised or lowered by 1°C at 18 day intervals

for the increasing and decreasing treatments, respectively. Temperatures were monitored twice daily during the experiment with standard thermometers and recorded hourly using three data loggers, one per treatment, and maintained within 0.3°C of the desired temperature during the experimental period.

Measurements and histology

During the experimental period, whole-body wet mass (BM) was recorded for each individual at 14 day intervals. Each octopus was carefully removed from its tank and quickly weighed in a beaker of tank water. At four intervals during the experiment [weeks 0, 5, 9 and 13, referred to as sample time (ST) 1, 2, 3 and 4, respectively], six individuals were selected randomly from each treatment and killed in chilled seawater. Animals taken at week 0 were subjected to 1 week of acclimation, but were not exposed to the experimental temperature regimes. The sex of each animal was determined by the presence (male) or absence (female) of the hectocotylus, and the dorsal mantle length (ML) was recorded. Subsequently, the reproductive organs were removed and weighed, and half the mantle muscle was removed by cutting along the dorsal longitudinal axis and fixed for histological analysis in a formalin–acetic acid–calcium chloride solution (FAACC). The muscle remained in FAACC for a minimum of 48 h, and was then transferred to 70% ethanol for at least 24 h before processing. Fixed muscle tissue from each individual was dehydrated in a graded ethanol series, cleared in toluene, infiltrated and impregnated with paraffin wax. Thin strips of muscle were cut along the longitudinal axis so that circular fibres were cut transversely [see fig. 1 in Pecl and Moltschaniwskyj (Pecl and Moltschaniwskyj, 1999) for more details] and embedded in wax using the Tissue-Tek embedding system. Sections were cut at 6 µm, stained with Mayer's haematoxylin and Young's eosin and mounted on slides with dibutylphthalate–polystyrene–xylene (DPX).

For each individual, muscle tissue measurements were taken at three positions along the mantle: anteriorly, mid-way along the ML and posteriorly. Using image analysis software and a light microscope (10× objective), five measurements of mantle thickness and the widths of five randomly selected muscle blocks were obtained at each region for each individual (Fig. 1). Muscle block width was taken as the distance between, but not including, radial muscle partitions, and in each case the maximum width was measured (see Pecl and Moltschaniwskyj, 1999). Circular muscle fibres are present in two structurally and functionally distinct forms within the cephalopod mantle: smaller mitochondria-poor (MP) fibres, which account for the bulk of the circular muscle, and larger mitochondria-rich (MR) fibres, which line the inner and outer edge of the circular muscle (Gosline and DeMont, 1985; Moltschaniwskyj, 1994). However, for *O. pallidus* it was noted that MR fibres were only present in the inner edge of the mantle. The diameters of 50 MP fibres and 20 MR fibres were measured at each region for each individual (100× objective with oil immersion), with the longest axis measured in every case. The mean diameter of the fibres at successive time intervals throughout the experiment was used to assess the relative contributions of hyperplasia and hypertrophy (i.e. if fibre size remains small while overall body mass increases it is assumed that new fibres are continuing to be generated) (see Martínez and Moltschaniwskyj, 1999).

Statistical analyses

A group growth curve was generated for the juvenile octopuses (1–156 days old), which were weighed prior to the manipulative experiment. As there were multiple y-values for each x-value, a weighted regression was performed based on the mean values of each 'multiple y' group, as detailed elsewhere (Sokal and Rohlf, 1995). A line of best fit for describing the growth was fitted, with linear, exponential and power growth equations tested; R^2 values were used to determine which equation best described the data. For the experimental animals (5–8 months old), group growth curves, using the above method, were generated for each temperature treatment based on the final mass of 18 individuals from each ST. An analysis of residual sum of squares (ARSS) (see Chen et al., 1992) was performed to test whether the growth curves for each treatment were statistically different. The ARSS requires that data from each treatment group be pooled, and a new curve fitted to the combined data. Individual growth curves for the 18 animals surviving until the end of the experiment (ST 4), and weighed at 14 day intervals, were also examined. Instantaneous growth rates (IGR), expressed in terms of the percentage increase in body size per day (% BM day^{-1}), were generated from each growth curve by multiplying the exponent of the equation by 100.

Three-way model I ANOVAs were used to test the significance of effects of temperature (treatment), age (ST) and mantle region on the four different measures of muscle tissue. For each combination of mantle region and individual, the mean of the dependent variable (mantle thickness, muscle block width, and MP

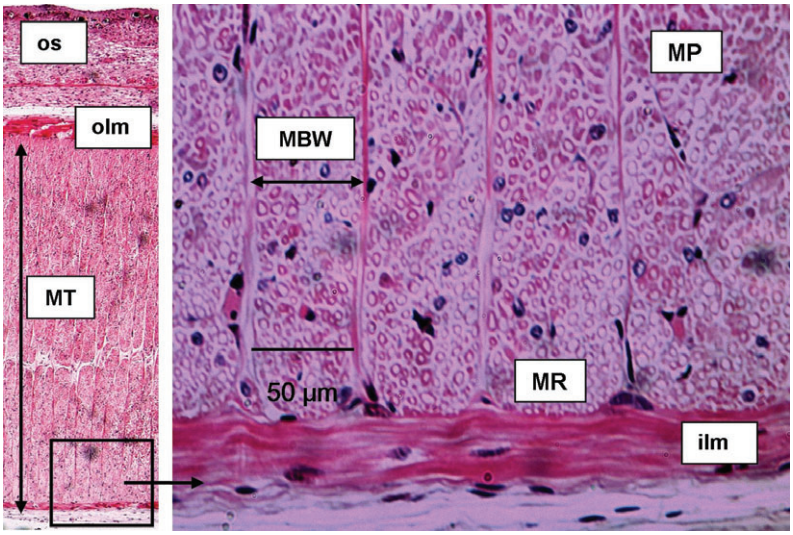

Fig. 1. A section of *Octopus pallidus* anterior mantle muscle, showing the four variables used to assess muscle growth: mantle thickness (MT), muscle block width (MBW), mitochondria-poor fibres (MP) and mitochondria-rich fibres (MR). Other features include: thick outer skin of mantle (os), outer longitudinal muscle (olm) and inner longitudinal muscle (ilm).

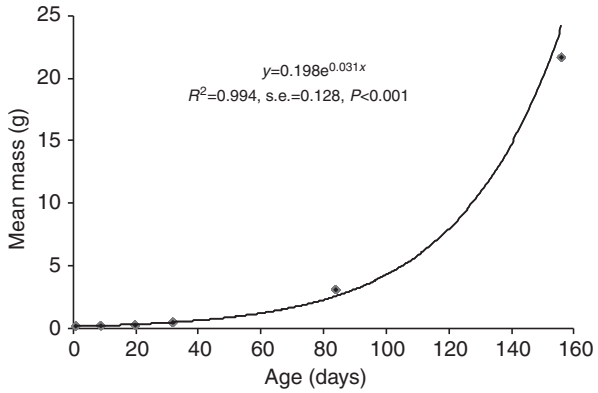

Fig. 2. Growth of juvenile *O. pallidus* aged 1–156 days and exposed to ambient temperatures. Each data point represents a mean value (for age at 1, 9, 20, 32, 84 and 156 days, n=109, 81, 79, 80, 110 and 76, respectively; total N=535).

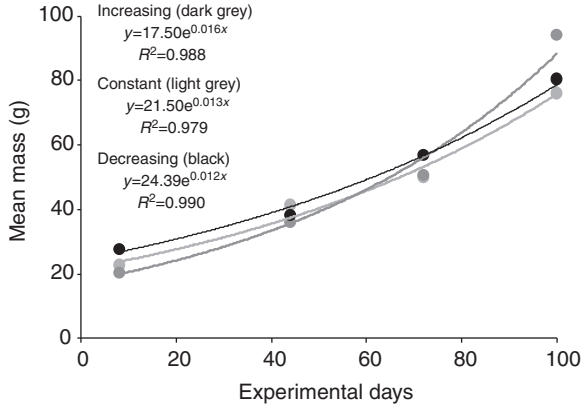

Fig. 3. Growth of *O. pallidus* under three different temperature regimes: increasing, decreasing and constant temperature. Each data point represents a mean value (n=6, N=24).

and MR fibre diameter) was used in the analyses, as single measurements within a region were not considered to be independent replicates. Where significance (P<0.05) was indicated, Tukey's HSD tests were used to identify the nature of the differences among groups. Size frequency distributions of all fibre measurements across the four ST were also explored.

Repro-somatic investment was compared between treatment groups by investigation of gonad mass *versus* mantle mass relationships. A two-way analysis of covariance (ANCOVA) was used to test the significance of differences in gonad mass between treatment groups, with mantle mass as a covariate. Sex was included as a factor in the analysis because of an observed difference in gonad size between males and females. No transformations were necessary. Mantle mass–length relationships were used to compare the condition of animals between treatment groups. ANCOVA was used to compare mantle mass among treatment groups, with mantle length as a covariate. To meet model assumptions the data were log-transformed. To enable comparison with the repro-somatic analyses, sex was also included as a factor.

RESULTS

Whole-body growth

At the group level, exponential growth was exhibited over the pre-experimental period from hatching to 156 days (Fig. 2). An exponential growth curve indicates that the IGR remained constant throughout the period investigated. The IGR of the pre-experimental juveniles was 3.1% BM day^{-1}. At the individual level, there was considerable variability in initial hatchling size (0.10–0.54 g), which was magnified throughout the growth period. For example, by day 84 animals weighed between 1.5 and 4.9 g and by day 156 animals weighed between 11 and 43 g.

At the group level, exponential growth was exhibited over the experimental period in all temperature treatments (increasing, decreasing and constant temperature) (Fig. 3). The ARSS indicated that three separate curves did not give a significantly better fit than a single curve fitted to the combined data ($F_{6,63}$=0.687, P=0.661). Thus, in terms of growth rate there was no significant difference between treatments over the experimental period.

All individuals that were maintained until the end of the experiment (n=18) also exhibited exponential growth curves during the experimental period. Individual IGRs ranged from 0.96 to 1.68% BM day^{-1} (Table 1). The relatively small differences in IGRs between individuals translated into large differences in size over time as a result of the nature of exponential growth. For example, two individuals from the decreasing temperature treatment, D5a and D5b, had very similar body masses at the start of the experiment, differing by only 1 g. However, the growth rate of 1.68% BM day^{-1} exhibited by D5b, when compared with 1.21% BM day^{-1} exhibited by D5a, produced an animal approximately 50 g heavier after 13 weeks (Table 1). In comparison to the pre-experimental juveniles, the experimental animals exhibited significantly slower IGRs (mean IGR: 1.3% BM day^{-1}).

Muscle tissue structure

Mean mantle thickness was not affected by temperature treatment (3-way ANOVA: $F_{2,180}$=2.09, P=0.12). The mantle in the posterior region was significantly thinner than the mantle in the mid or anterior regions, but the extent of these differences was modified by ST as

Table 1. Instantaneous growth rate (IGR) and pre- and post-experimental mass (g) for all *Octopus pallidus* individuals kept for the duration of the experimental period

Treatment, tank and individual	IGR (% BM day^{-1})	Pre-mass (g)	Post-mass (g)
C1b	1.06	27.44	79.96
C2a	1.32	13.92	58.10
C2b	1.45	21.6	98.81
C3d	1.39	24.93	97.11
C4a	1.09	28.84	84.34
C5c	0.96	14.17	38.33
D1b	1.36	13.40	52.95
D2b	1.35	20.73	79.22
D3d	1.48	12.30	55.47
D4d	1.48	11.92	53.83
D5a	1.21	28.49	95.71
D5b	1.68	27.47	145.89
I1b	1.42	17.24	72.79
I2a	1.42	23.13	99.90
I2c	1.43	31.36	130.97
I3b	1.35	20.40	86.16
I4b	1.31	12.43	46.47
I5c	1.65	25.31	128.87

In the first column, the capital letter describes the temperature treatment (C, constant; D, decreasing, and I, increasing), the number describes the tank the individual was sampled from, and the lower-case letter represents the individual.

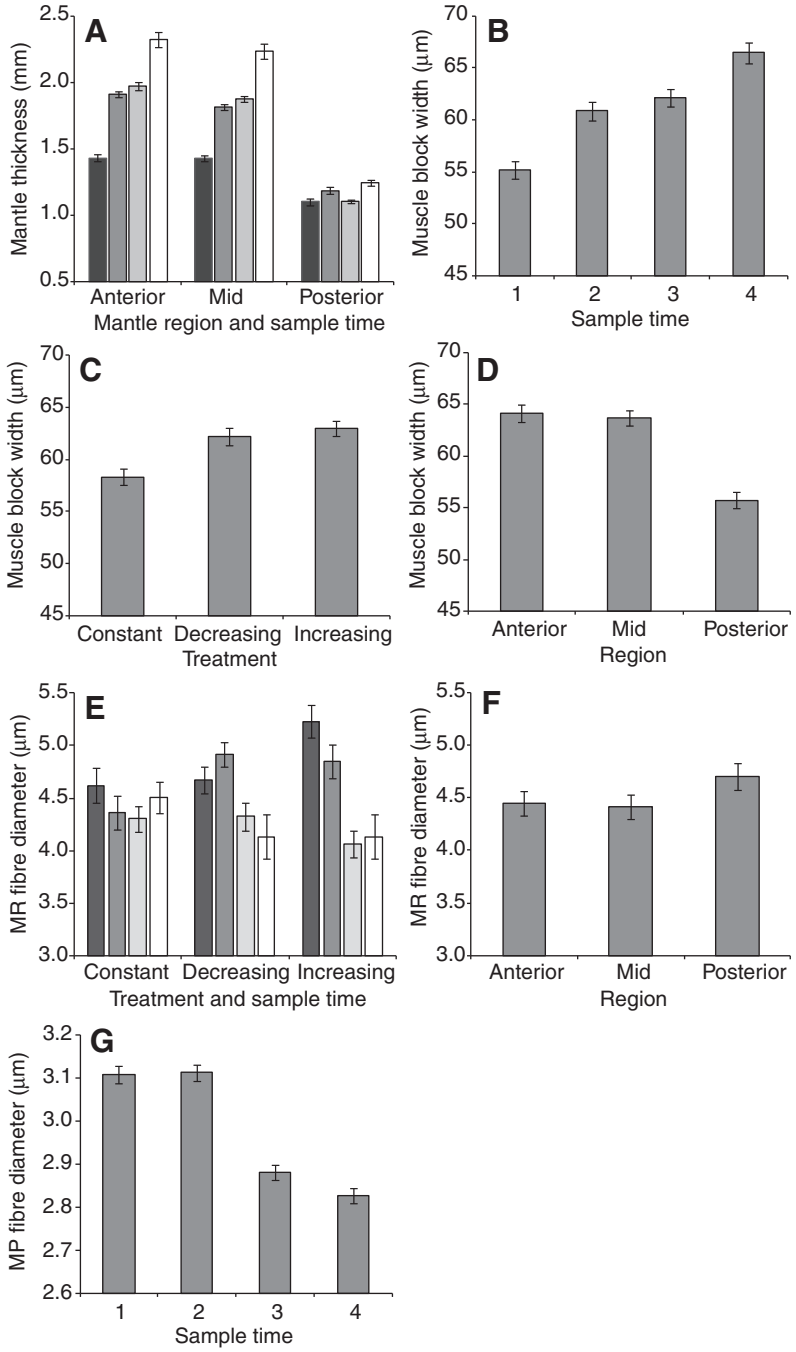

Fig. 4. Muscle tissue structure in relation to temperature treatment (constant, decreasing, increasing), sample time (ST 1–4) and mantle region (anterior, mid, posterior). Only significant results are presented, and columns represent the mean ± s.e. (A) Mantle thickness at each ST (indicated by dark grey, mid-grey, light grey and white columns for ST 1–4, respectively), within each region; (B) muscle block width at each ST; (C) muscle block width for each treatment; (D) muscle block width at each region; (E) mitochondria-rich (MR) fibre diameter at each ST (indicated by dark grey, mid-grey, light grey and white columns for ST 1–4, respectively) and for each treatment; (F) MR fibre diameter at each region; (G) mitochondria-poor (MP) fibre diameter at each ST.

evidenced by a significant interaction effect between ST and region (3-way ANOVA: $F_{6,180}$=5.91, P<0.001) (Fig. 4A). *Post hoc* analysis indicated that posterior mantle thickness was similar for each ST, but the anterior and mid-mantle regions became increasingly thicker at each consecutive ST from 1 to 4 (Fig. 4A). Size frequency distributions of all fibre measurements were similar between each ST, which were generally bell shaped with a slight negative skew.

Mean muscle block width varied significantly between temperature treatment groups, STs and regions (3-way ANOVA: treatment, $F_{2,180}$=4.17, P=0.017; ST, $F_{3,180}$=10.90, P<0.001; region, $F_{2,180}$=14.97, P<0.001), and each of these factors produced an effect independent of the other factors (3-way ANOVA: $F_{12,180}$=0.50, P=0.90). Muscle block width became larger at each consecutive ST from 1 to 4 (Fig. 4B), and was significantly wider in individuals exposed to increasing and decreasing temperatures, compared with constant temperatures (Fig. 4C). Muscle block widths were similar for the anterior and mid-mantle regions, but were significantly smaller in the posterior region (Fig. 4D).

Mean MP fibre diameter differed between STs (3-way ANOVA: $F_{3,180}$=11.50, P<0.001); however, the size of these fibres was not influenced by temperature treatment or mantle region (3-way ANOVA: treatment, $F_{2,180}$=0.97, P=0.37; region, $F_{2,180}$=1.99, P=0.13) and there were no significant interaction effects (3-way ANOVA: $F_{12,180}$=0.59, P=0.84). Fibre diameters were similar in individuals at STs 1 and 2 and at STs 3 and 4, with the latter being significantly smaller (Fig. 4G). Thus, mean MP fibre diameter decreased over the experimental period across all levels of treatment and region. Mean MR fibre diameter varied significantly between

mantle regions and ST (3-way ANOVA: ST, $F_{3,180}$=11.7, P<0.001; region, $F_{2,180}$=4.24, P=0.016), and there was a significant interaction effect between treatment and ST (3-way ANOVA: $F_{6,180}$=3.423, P=0.003). In individuals exposed to constant temperature, MR fibre diameter did not change significantly over the experimental period, while individuals subject to either decreasing or increasing temperatures had significantly smaller MR fibres at STs 3 and 4 than at STs 1 and 2 (Fig. 4E). Fibre diameter was significantly larger in the posterior region than in both anterior and mid regions (Fig. 4F).

Repro-somatic investment

The linear relationship between gonad mass and mantle mass was examined to assess relative repro-somatic investment between treatment groups and the sexes (Fig. 5). For all combinations of treatment and sex, except in females in the constant treatment group, there was a strong relationship between mantle mass and gonad mass, with mantle mass accounting for >73% of the variability in gonad mass in all cases. The three-way interaction between treatment, sex and the covariate (mantle mass) was significant (2-way ANCOVA: $F_{1,40}$=4.85, P=0.033), indicating that the slopes describing the increase in gonad material per unit of mantle mass differed depending on the combination of treatment and sex. A comparison of slopes suggests that, in all treatments, males were developing gonads at a faster rate relative to mantle mass than females (Fig. 5). It also appears that for a given mantle mass, both males and females exposed to decreasing temperatures were investing substantially more in gonad material than animals exposed to increasing temperatures. Despite the increased investment in gonad growth exhibited by both males and females from the decreasing temperature treatment group, there did not appear to be a corresponding decline in somatic condition of the mantle. There were no differences between treatment groups (2-way ANCOVA: $F_{2,65}$=2.876, P=0.064) or the two sexes (2-way ANCOVA: $F_{1,65}$=0.094, P=0.76) in terms of mantle mass–length relationships.

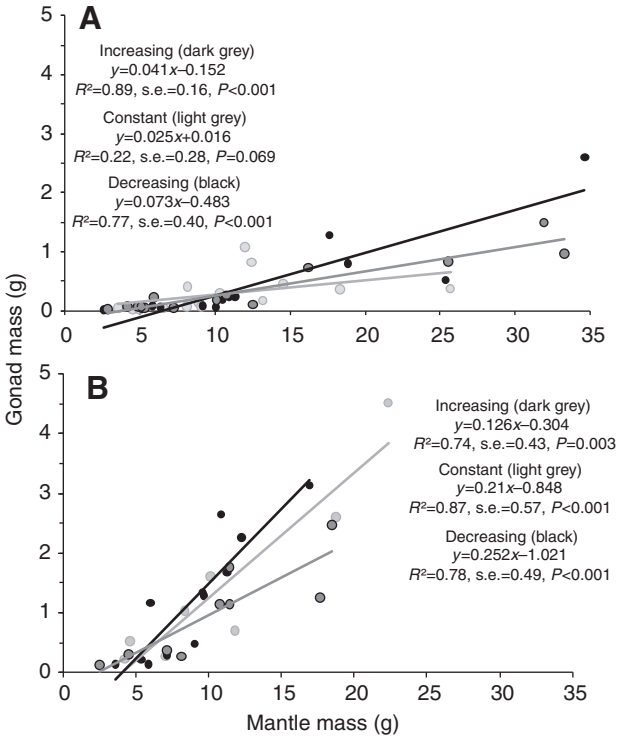

Fig. 5. Repro-somatic investment for (A) female and (B) male *O. pallidus* under three different temperature regimes: constant, increasing and decreasing.

DISCUSSION

Octopus pallidus exhibited exponential growth over the two age periods examined in this study. IGRs of the older experimental individuals did not appear to be declining, but were sustained at a constant rate, despite the fact that they were well into the adult phase and approaching maturity or fully mature at the end of the experiment. However, the growth rate in the early growth phase was nearly three times faster than the growth rate during the experimental period, indicating a two-phase double-exponential growth pattern. Although the pre-experimental and experimental animals were exposed to ambient and controlled temperature regimes, respectively, which may have influenced the growth pattern, the ambient temperature range was within the temperature ranges of all three controlled treatments. Two-phase growth is well documented in octopus and other cephalopod species raised in captivity, with growth, best described by a power function, typically reported in the second phase (e.g. DeRusha et al., 1987; Forsythe and Hanlon, 1988; Forsythe and Toll, 1991), although logarithmic growth in the second phase has also been reported for octopus species (Cortez et al., 1999). Double-exponential growth curves have only been observed in squid (Hatfield et al., 2001; Natsukari et al., 1993). The IGR of juveniles in this study was significantly higher than that recorded in a previous study on juvenile *O. pallidus* held at similar temperatures (mean: 1.57% BM day^{-1}) (Leporati et al., 2007). Growth rates reported for juveniles of other holobenthic octopus species vary greatly [e.g. 2.23% BM day^{-1} (Briceño et al., 2010); 7.4% BM day^{-1} (Forsythe, 1984)]; however, comparisons are difficult because of different culture conditions and thermal optima of each species. In the wild, *O. pallidus* attains a maximum mass of approximately 1 kg and typically lives for about 12 months, with a maximum of 19 months for males and 16 months for females (Leporati et al., 2008b). These life spans fit the growth rates demonstrated in this study. Hypothetically, if growth continued exponentially at 1.3% BM day^{-1} from the end of the experiment (aged 8 months), an individual weighing 80 g would reach 800 g at approximately 14 months of age. The largest individual in the study, growing at 1.6% BM day^{-1}, would reach 1 kg at approximately 11 months of age. Furthermore, if a day-old hatchling, which weighs about 0.2 g, grew exponentially at 1.6% BM day^{-1}, it would only weigh 2.3 g after 156 days or 64 g after 12 months, supporting the suggestion of substantially faster growth during the early phase of the life cycle. Interestingly, modelled juvenile growth trajectories of *O. pallidus*, based on size-at-age data of wild immature females, suggest that *O. pallidus* populations comprise individuals with single-phase exponential growth and others with two-phase growth, with the proportions of each growth pattern influenced predominantly by food availability and the inherent growth capacity of the individual (André et al., 2009b). This may explain the differences in growth rates between this study and that of Leporati and colleagues (Leporati et al., 2007).

Considerable variability in size-at-age was observed for *O. pallidus* from hatching to 8 months old, even in individuals experiencing identical thermal conditions (e.g. in the decreasing temperature treatment, 8 month old animals weighed between 52 and 145 g). Other studies have also noted significant variation in initial size at hatching, growth and size-at-age between individual octopuses reared under identical laboratory conditions (Forsythe, 1984; Forsythe and Hanlon, 1985; Leporati et al., 2007). While

growth and size-at-age at an individual level can be highly influenced by environmental variables such as temperature (Forsythe and Hanlon, 1988; Segawa and Nomoto, 2002) and food availability (O'Dor et al., 1980), because of the nature of exponential growth, inherent growth plasticity and hatchling size heterogeneity can also lead to considerable differences in size over time. Modelled growth projections of *O. pallidus* suggest that inherent growth capacities have a strong influence on size-at-age, as does initial hatchling size (André et al., 2009b). Initial hatchling size was also found to influence growth rate in laboratory-raised juvenile *O. pallidus*, although the effects were secondary to temperature (Leporati et al., 2007).

Interestingly, there was no detectable effect of temperature on somatic growth of *O. pallidus* at the whole-animal level. Within the limits of thermal tolerance for a species, the general response of marine ectotherms to higher temperatures is a higher growth rate (Boyle and Boletzky, 1996; Wood and O'Dor, 2000). On the basis that cephalopods have metabolic rates that rise or drop directly with temperature with very little tendency for compensation, Forsythe suggested that small differences in the temperatures experienced by juvenile cephalopods can produce significantly different growth rates and, consequently, variable size-at-age in later life (Forsythe, 2004; Forsythe, 1993). While many studies, both laboratory and field based, have provided examples of the dramatic effect of temperature on cephalopod growth (e.g. Forsythe et al., 2001; Hatfield, 2000; Segawa and Nomoto, 2002; Villanueva, 2000), including *O. pallidus* (Leporati et al., 2007), this effect has always been linked to the juvenile growth phase. It is likely that the second phase of growth is far less temperature dependent (Forsythe and Van Heukelem, 1987). For example, elevated temperatures caused a dramatic increase in growth rate during the exponential growth phase of *O. bimaculoides*, but the effect was diminished as the octopus entered the slower adult power growth phase (Forsythe and Hanlon, 1988). Similarly, it appears that temperature does not affect the growth rate of sub-adult and adult *O. pallidus* at the whole-body level. In contrast, muscle tissue growth in *O. pallidus* was modified by temperature. Animals under increasing and decreasing temperature regimes exhibited a change in the relative balance of hyperplasia and hypertrophy in MR muscle over time, while this balance remained unchanged for animals under constant temperature. This result, while not associated with a change in growth rate at the whole-body level, may reflect a different effect of fluctuating temperature on muscle growth. Pecl and Moltchaniwskyj found that captivity altered the muscular growth mechanisms (including fewer small muscle fibres) in adult *I. pygmaeus*, rather than simply changing the physiological rate of growth (Pecl and Moltchaniwskyj, 1999). It is possible that constant temperatures, which were used in the Pecl and Moltchaniwskyj study (Pecl and Moltchaniwskyj), may produce an unnatural effect on muscle growth. Animals exposed to increasing or decreasing temperatures also had larger muscle blocks compared with animals from constant temperatures. However, the effect of temperature was not modified by ST, indicating that the difference was evident before any changes associated with experimental temperature regimes were implemented.

This is the first report of ongoing hyperplasia as the mechanism facilitating non-asymptotic growth in an octopus species. Mean MP fibre diameter remained small in all regions of the mantle and under all temperature regimes, indicating that new MP fibres were produced continuously over time. Given that MP fibres make up the bulk of the mantle muscle, they would be important for maintaining continued growth of the whole animal. Notably, a marked decrease in mean fibre size occurred approximately halfway through the experiment, indicating a change in the relative contribution of the two muscle growth processes. It appears that as the animals grow, not only is new fibre production sustained but also hyperplasia increasingly becomes the dominant process of muscle growth. A similar pattern was observed in the MR fibres, for individuals exposed to increasing and decreasing water temperatures. The increasingly important role of hyperplasia relative to hypertrophy over time is particularly interesting given that adult growth rates did not change, and were considerably slower compared with the early growth phase. It is likely that as the animal grows larger, the existing balance of hypertrophy and hyperplasia is not sufficient to sustain exponential growth at a constant rate. Furthermore, by keeping fibre size low the efficiency of cellular processes is maximised; continued hyperplasia, therefore, may also confer greater energetic advantages. Although hyperplasia was continuous throughout the lifespan of *I. pygmaeus* and *Photololigo* sp., in contrast, proportionally fewer new fibres were produced in larger animals (Moltschaniwskyj, 1994; Pecl and Moltschaniwskyj, 1997). Interestingly, the last two species are multiple spawners unlike the terminally spawning *O. pallidus*; different energetic demands, therefore, may explain such differences in the muscle fibre dynamics.

Exposure to seasonally increasing or decreasing temperatures, while not affecting somatic growth rate, did affect the allocation of energy to reproduction, reflected in differences in the rate of gonad development. Although gonad development, relative to body size, was significantly faster in males than in females, the effect of temperature was evident in both sexes. Maturation in wild *O. pallidus* has little relationship to age and is primarily size dependent, suggesting that maturation is influenced by inherent individual variability and abiotic factors such as temperature (Leporati et al., 2008a). Forsythe noted the tendency among shallow-water cephalopods to spawn in colder temperatures, so that hatching young would be exposed to continually increasing temperatures for the first few months of life when growth is rapid (Forsythe, 1993). If wild *O. pallidus* does display a rapid growth phase in early life, it may explain why individuals growing under decreasing water temperatures appeared to direct proportionally more energy to gonad growth than animals from the increasing temperature treatment. Animals experiencing decreasing temperatures may have 'sped up' the rate of maturation, or animals exposed to increasing temperatures may have 'slowed down' the normal rate of maturation. Effectively, the process of maturation appeared to be occurring at a rate such that spawning would take place in colder temperatures, thus conferring the warm-temperature advantage to the offspring. *Octopus pallidus* hatchlings grew faster and larger when they hatched at higher summer temperatures (18°C) compared with lower spring temperatures (14°C), supporting this suggestion (Leporati et al., 2007). While *O. pallidus* individuals spawn year round in the wild, there are seasonal trends in reproductive scheduling, with peak reproductive investment in spring and summer for females and males, respectively (Leporati et al., 2008a), suggesting an optimal spawning period in late summer/early autumn. This is contrary to our conclusions as *O. pallidus* eggs have an incubation period of about 4 months (i.e. if octopus spawn in March the eggs would hatch in July or mid-winter). However, if peak spawning occurred in late autumn (May) the eggs would hatch in spring (September), which would support our conclusions.

Although individuals exposed to decreasing temperatures allocated more energy to reproduction than their counterparts, it was not at the expense of overall growth rate or condition. Other studies have revealed no evidence of a trade-off between reproduction and growth

or condition in cephalopods (e.g. Ho et al., 2004). It is accepted that cephalopods direct energy into growth rather than storage (Semmens, 1998), and it is probable that energy for gonad growth is predominantly derived directly from food (Moltschaniwskyj and Semmens, 2000). Animals were fed *ad libitum* in this study and it can be assumed that food supply did not limit growth. For terminal spawners, an individual that directs more energy to reproduction without a corresponding decline in growth rate may do so at the expense of a longer life span. However, unlike squid and cuttlefish octopus have a much heavier somatic investment in the arms and arm crown and much less mantle muscle (Trueman and Packard, 1968). It is possible that the octopus simply lost condition in the arms rather than the mantle; however, this was not measured.

This study has provided further support for the developing view of cephalopods as short-lived organisms exhibiting rapid and continuous growth. *Octopus pallidus* grew exponentially from hatching to 8 months, but substantially slower in the post-juvenile phase, suggesting a two-phase growth pattern. However, two-phase growth needs to be examined in the wild to validate this assumption. Previous research has emphasised the critical impact of temperature on juvenile octopus growth, with little known about the impact of temperature on adult growth. Although muscle tissue structure was affected by temperature, overall growth of adult *O. pallidus* was not, suggesting that individuals had shifted to a growth phase that was not temperature dependent. Relationships between feeding and food conversion rate, temperature and growth have been examined in juvenile octopus (André et al., 2008), and it would be interesting to examine such relationships in adult octopus to gain further insight into the underlying factors affecting growth in adults. We report for the first time the ongoing recruitment of new muscle fibres or hyperplasia in an octopus species. Interestingly, hyperplasia was not only found to be ongoing but also became the increasingly dominant process of muscle growth (relative to hypertrophy) over time, which has not been observed before in a cephalopod species. Another key result is that repro-somatic investment varied according to temperature regime, with animals exposed to decreasing temperatures appearing to mature at a faster rate; this supports the view that cephalopods tend to spawn in colder months so that the offspring are exposed to warming temperatures. This research furthers our understanding of some fundamental aspects of octopus biology and physiology, and will thus have important applications for the sustainable management of commercial species, which increasingly constitute large and important fisheries, and aquaculture research. This research also provides the basis for further examination of cephalopod growth and the effect of temperature on growth and maturity.

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REFERENCES

Alford, R. A. and Jackson, G. D. (1993). Do cephalopods and larvae of other taxa grow asymptotically? *Am. Nat.* **141**, 717-728.

André, J., Pecl, G. T., Semmens, J. M. and Grist, E. P. M. (2008). Early life-history processes in benthic octopus: relationships between temperature, feeding, food conversion, and growth in juvenile *Octopus pallidus*. *J. Exp. Mar. Biol. Ecol.* **354**, 81-92.

André, J., Grist, E. P. M., Semmens, J. M., Pecl, G. T. and Segawa, S. (2009a). Effects of temperature on energetics and the growth pattern of benthic octopuses. *Mar. Ecol. Prog. Ser.* **374**, 167-179.

André, J., Pecl, G. T., Grist, E. P. M., Semmens, J. M., Haddon, M. and Leporati, S. C. (2009b). Modelling size-at-age in wild immature female octopus: a bioenergetics approach. *Mar. Ecol. Prog. Ser.* **384**, 159-174.

Boyle, P. R. and Boletzky, S. v. (1996). Cephalopod populations: definition and dynamics. *Philos. Trans. R. Soc. Lond. B* **351**, 985-1002.

Briceño, F., Mascaró, M. and Rosas, C. (2010). Energy demand during exponential growth of *Octopus maya*: exploring the effect of age and weight. *ICES J. Mar. Sci.* **67**, 1501-1508.

Chen, Y., Jackson, D. A. and Harvey, H. H. (1992). A comparison of von Bertalanffy and polynomial functions in modelling fish growth data. *Can. J. Fish. Aquat. Sci.* **49**, 1228-1235.

Cortez, T., González, A. and Guerra, A. (1999). Growth of cultured *Octopus mimus* (Cephalopoda, Octopodidae). *Fish. Res.* **40**, 81-89.

DeRusha, R. H., Forsythe, J. W. and Hanlon, R. T. (1987). Laboratory growth, reproduction and life-span of the Pacific Pygmy Octopus, *Octopus digueti*. *Pac. Sci.* **41**, 104-121.

Doubleday, Z. A., Pecl, G. T., Semmens, J. M. and Danyushevsky, L. (2008). Stylet elemental signatures indicate population structure in a holobenthic octopus species, *Octopus pallidus*. *Mar. Ecol. Prog. Ser.* **371**, 1-10.

Forsythe, J. (2004). Accounting for the effect of temperature on squid growth in nature: from hypothesis to practice. *Mar. Freshw. Res.* **55**, 331-339.

Forsythe, J. W. (1984). *Octopus joubini* (Mollusca:Cephalopoda): a detailed study of growth through the full life cycle in a closed seawater system. *J. Zool.* **202**, 393-417.

Forsythe, J. W. (1993). A working hypothesis of how seasonal temperature change may impact the field growth of young cephalopods. In *Recent Adances in Cephalopod Fisheries Biology* (ed. T. Okutani, R. K. O'Dor and T. Kubodera), pp. 133-143. Tokyo: Tokai University Press.

Forsythe, J. W. and Hanlon, R. T. (1985). Aspects of egg development, post-hatching behaviour, growth and reproductive biology of *Octopus burryi* Voss, 1950 (Mollusca:Cephalopoda). *Vie Milieu* **35**, 273-282.

Forsythe, J. W. and Hanlon, R. T. (1988). Effect of temperature on laboratory growth, reproduction and life span of *Octopus bimaculoides*. *Mar. Biol.* **98**, 369-379.

Forsythe, J. W. and Toll, R. B. (1991). Clarification of the western Atlantic ocean pygmy octopus complex: the identity and life history of *Octopus joubini* (Cephalopoda: Octopodinae). *Bull. Mar. Sci.* **49**, 88-97.

Forsythe, J. W. and Van Heukelem, W. F. (1987). Growth. In *Cephalopod Life Cycles*, Vol. 2 (ed. P. R. Boyle), pp. 135-156. London: Academic Press.

Forsythe, J. W., Walsh, L. S., Turk, P. E. and Lee, P. G. (2001). Impact of temperature on juvenile growth and age at first egg-laying of the Pacific reef squid *Sepioteuthis lessoniana* reared in captivity. *Mar. Biol.* **138**, 103-112.

Gosline, J. M. and DeMont, M. E. (1985). Jet-propelled swimming in squids. *Sci. Am.* **252**, 74-79.

Hatfield, E. (2000). Do some like it hot? Temperature as a possible determinant of variability in the growth of the Patagonian squid, *Loligo gahi* (Cephalopoda: Loliginidae). *Fish. Res.* **47**, 27-40.

Hatfield, E., Hanlon, R., Forsythe, J. and Grist, E. (2001). Laboratory testing of a growth hypothesis for juvenile squid *Loligo pealeii* (Cephalopoda: Loliginidae). *Can. J. Fish. Aquat. Sci.* **58**, 845-857.

Ho, J., Moltschaniwskyj, N. A. and Carter, C. G. (2004). The effect of variability in growth on somatic condition and reproductive status in the southern calamary *Sepioteuthis australis*. *Mar. Freshw. Res.* **55**, 423-428.

Jackson, G. D. and O'Dor, R. K. (2001). Time, space and the ecophysiology of squid growth, life in the fast lane. *Vie Milieu* **51**, 205-215.

Leporati, S. C., Pecl, G. T. and Semmens, J. M. (2007). Cephalopod hatchling growth: the effects of initial size and seasonal temperatures. *Mar. Biol.* **151**, 1375-1383.

Leporati, S. C., Pecl, G. T. and Semmens, J. M. (2008a). Reproductive status of *Octopus pallidus*, and its relationship to age and size. *Mar. Biol.* **155**, 375-385.

Leporati, S. C., Semmens, J. M. and Pecl, G. T. (2008b). Determining the age and growth of wild octopus using stylet increment analysis. *Mar. Ecol. Prog. Ser.* **367**, 213-222.

Martínez, P. and Moltschaniwskyj, N. A. (1999). Description of growth in the tropical cuttlefish *Sepia elliptica* using muscle tissue. *J. Mar. Biol. Assoc. UK* **79**, 317-321.

McGrath Steer, B. (2003). Flexibility in reproductive strategies of squid: a case study using the arrow squid *Nototodarus gouldi* from southeastern Australia. PhD thesis, University of Tasmania, Hobart, Australia.

Moltschaniwskyj, N. A. (1994). Muscle tissue growth and muscle fibre dynamics in the tropical loliginid squid *Photololigo* sp. (Cephalopoda:Loliginidae). *Can. J. Fish. Aquat. Sci.* **51**, 830-835.

Moltschaniwskyj, N. A. (2004). Understanding the process of growth in cephalopods. *Mar. Freshw. Res.* **55**, 379-386.

Moltschaniwskyj, N. A. and Semmens, J. M. (2000). Limited use of stored energy reserves for reproduction by the tropical loliginid squid *Photololigo* sp. *J. Zool.* **251**, 307-313.

Natsukari, Y., Mukai, H., Nakahama, S. and Kubodera, T. (1993). Age and growth estimation of a gonatid squid *Berryteuthis magister*, based on statolith microstructure (Cephalopoda: Gonatidae). In *Recent Advances in Fisheries Biology* (ed. T. Okutani, R. K. O'Dor and T. Kubodera), pp. 351-364. Tokyo: Tokai University Press.

O'Dor, R. K., Durward, R. D., Vessey, E. and Amaratunga, T. (1980). Feeding and growth in captive squid, *Illex illecebrosus,* and the influence of food availability on growth in the natural population. *ICNAF Sel. Pap.* **6**, 1-21.

Pecl, G. T. and Moltschaniwskyj, N. A. (1997). Changes in muscle structure associated with somatic growth in *Idiosepius pygmaeus*, a small tropical cephalopod. *J. Zool.* **242**, 751-764.

Pecl, G. T. and Moltschaniwskyj, N. A. (1999). Somatic growth processes: how are they modified in captivity? *Proc. R. Soc. Lond. B* **266**, 1133-1139.

Preuss, T., Lebaric, Z. N. and Gilly, W. F. (1997). Post-hatching development of circular mantle muscles in the squid *Loligo opalescens*. *Biol. Bull.* **192**, 375-387.

Rowlerson, A., Mascarello, F., Radaelli, G. and Veggetti, A. (1995). Differentiation and growth of muscle in the fish *Sparus aurata* (L): II. Hyperplastic and hypertrophic

growth of lateral muscle from hatchling to adult. *J. Muscle Res. Cell Motil.* **16**, 223-236.

Segawa, S. and Nomoto, A. (2002). Laboratory growth, feeding, oxygen consumption and ammonia excretion of *Octopus ocellatus*. *Bull. Mar. Sci.* **71**, 801-813.

Semmens, J. (1998). An examination of the role of the digestive gland of two loliginid squids, with respect to lipid: storage or excretion? *Proc. R. Soc. Lond. B* **265**, 1685-1690.

Semmens, J. M., Pecl, G. T., Villanueva, R., Jouffre, D., Sobrino, I., Wood, J. B. and Rigby, P. R. (2004). Understanding octopus growth: patterns, variability and physiology. *Mar. Freshw. Res.* **55**, 367-377.

Sokal, R. R. and Rohlf, F. J. (1995). *Biometry*. New York: W. H. Freeman and Company.

Stranks, T. N. (1996). Biogeography of *Octopus* species (Cephalopoda: Octopodidae) from southeastern Australia. *Am. Malacol. Bull.* **12**, 145-151.

Trueman, E. R. and Packard, A. (1968). Motor performances of some cephalopods. *J. Exp. Biol.* **49**, 495.

Villanueva, R. (2000). Effect of temperature on statolith growth of the European squid *Loligo vulgaris* during early life. *Mar. Biol.* **136**, 449-460.

Weatherley, A. H. (1990). Approaches to understanding fish growth. *Trans. Am. Fish. Soc.* **119**, 662-672.

Weatherley, A. H. and Gill, H. S. (1987). *The Biology of Fish Growth*. London: Academic Press.

Wood, J. B. and O'Dor, R. K. (2000). Do larger cephalopods live longer? Effects of temperature and phylogeny on interspecific comparisons of age and size at maturity. *Mar. Biol.* **136**, 91-99.