

Effect of water temperature on reproductive development of *Benedenia seriolae* (Monogenea: Capsalidae) from *Seriola lalandi* in Australia

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ABSTRACT: The monogenean *Benedenia seriolae* (Yamaguti, 1934) Meserve, 1938 is a major pathogen of farmed yellowtail kingfish *Seriola lalandi* in South Australia. To control parasite populations in commercial farms, an understanding of the effect of water temperature on parasite development is vital. This study investigated the effect of water temperature (14, 18, 22 and 26 ± 0.5°C) on development, growth rates and age at sexual maturity of *B. seriolae*. Five distinct developmental stages, defined by the progressive development of reproductive organs, were used to describe development of *B. seriolae* from recently invaded larvae to sexually mature adults. Parasite age at the first sign of sexual maturity (ability to lay eggs) was strongly influenced by water temperature and was attained at 41, 24, 16 and 14 d post infection (p.i.) at 14, 18, 22 and 26 ± 0.5°C respectively. Four parameters (parasite total length, maximum parasite width, accessory sclerite length and anterior hamulus length) were examined for suitability as an index of parasite age. Growth rates for each parameter increased with water temperature; however, mean anterior hamulus length was identified as the most reliable index of parasite age. Equations derived from these data can be used to estimate parasite age and time to sexual maturity at water temperatures ranging from 14 to 26°C. The equations provide a simple tool to assist implementation of strategic treatment plans for *B. seriolae* infections in commercial kingfish farms, not only in South Australia but in other localities worldwide where *S. lalandi* is farmed.

KEY WORDS: Monogenea · Capsalidae · Sea-cage aquaculture · *Benedenia seriolae* · Water temperature · Growth rates · Sexual maturity · Strategic treatment

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INTRODUCTION

Marine finfish aquaculture is a fast-growing sector globally, responsible for high-value fish products. Pathogens can have expensive consequences for finfish farms, not only owing to their effect on fish health, reduced growth, marketability and even mortalities, but also to the increased labour and infrastructure

costs necessary to manage infections. Monogeneans are significant parasites in *Seriola* spp. culture in Japan, New Zealand and Australia because they can proliferate rapidly due to their direct, single-host life cycle (Whittington 2005). In South Australia, 2 monogenean species are problematic in sea-cage culture of yellowtail kingfish *Seriola lalandi*: *Zeuxapta seriolae* (Heteraxinidae), a blood feeding polyopisthocotylean

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of the gills, and *Benedenia seriolae* (Capsalidae), a skin feeding monopisthocotylean parasitising the body surfaces. Information on egg laying, embryonation, hatching, parasite growth and development rates, especially age at sexual maturity, is vital for the development of strategies to manage and control parasite populations in farmed kingfish in sea-cages. Tubbs et al. (2005) determined the effect of temperature on *in vitro* fecundity, egg hatching and time to sexual maturity for *Z. seriolae* and *B. seriolae* from infected *S. lalandi* in New Zealand. Mooney et al. (2006) presented data for an *in vivo* egg laying rhythm in *Z. seriolae* from *S. lalandi* culture in South Australia. In the present study, we present *in vivo* data on the effect of water temperature on *B. seriolae* development, and determine the age at which sexual maturity is attained from experimental infections of *S. lalandi*. Experiments were run at 14, 18, 22 and $26 \pm 0.5^\circ\text{C}$ to represent local water temperatures that farmed *S. lalandi* experience in Spencer Gulf, South Australia. We discuss the application of these data to the effective management of *B. seriolae* infections in *S. lalandi* farms in South Australia.

MATERIALS AND METHODS

Source of fish. In February 2004, 84 *Seriola lalandi* (weight range: 80 to 200 g; fork length range: 18 to 22 cm) from a commercial fish farm near Whyalla, Spencer Gulf, South Australia, were transported to the South Australian Aquatic Sciences Centre (SAASC), Adelaide. Until September 2004, fish were maintained in a 2100 l flow-through tank at 16°C and fed daily on a commercial kingfish diet (3 mm Skretting classic HS). During September 2004, fish were transferred to a 2100 l flow-through tank at ambient temperature (approximately 21°C) and fed daily. Four days before infection with *Benedenia seriolae* larvae (see below), groups of approximately 40 fish were bathed in 160 l of dechlorinated tap water for 5 min to ensure they were free of any existing *B. seriolae* infection (Chambers & Ernst 2005). After bathing, 43 and 41 fish were transferred into 2 separate temperature-controlled, flow-through tanks containing 600 l of seawater (salinity 41 ppt, flow rate 1200 l h^{-1} with continual aeration) for 4 d acclimation before experimental infection.

Water temperatures of 14, 18, 22 and 26°C were selected to represent the annual temperature range experienced by farmed *Seriola lalandi* in Spencer Gulf, South Australia (where, however, water temperatures may drop below 14°C for periods in winter). To avoid cross contamination between adjacent tanks in concurrent experiments, separate equipment was used for each experimental tank. After each sampling event, all

bathing tanks, nets and filters were cleaned thoroughly with hot tap water and left to air dry to kill any *Benedenia seriolae* eggs (Ernst et al. 2005). To prevent water transfer between tanks from fish splashing during feeding, plastic curtains were erected to isolate adjacent tanks. Water temperature $\pm 0.5^\circ\text{C}$ of the target temperature was maintained via water inflow from the SAASC system and was measured in each tank every minute using a 4-channel data-logging thermometer (Sper Scientific).

Initial source of parasites. Using fine forceps, approximately 400 live, adult *Benedenia seriolae* were carefully removed from the skin of several infected *Seriola lalandi* at a commercial farm near Whyalla, Spencer Gulf, South Australia. Parasites were transferred into 250 ml plastic jars containing approximately 100 ml of Millipore filtered ($0.2 \mu\text{m}$) seawater (FSW, salinity 41 ppt) and maintained at $20 \pm 1^\circ\text{C}$ during the 5 h journey to SAASC, during which time approximately 15300 eggs ($\sim 7.65 \text{ eggs parasite}^{-1} \text{ h}^{-1}$) were laid *in vitro*.

Experimental infections. Eggs laid *in vitro* were incubated in small Perspex wells (1 ml volume, internal diameter 9 mm, see Kearn 1973) immersed in a glass dish (volume 30 ml, 4 cm diameter, 3 cm depth) containing filtered seawater (FSW) at $20 \pm 0.1^\circ\text{C}$ and exposed to a 12:12 h light:dark regime (light on 06:00 h, light off 18:00 h). Each dish was sealed with a glass plate and water changes were made twice daily. After 7 d, glass plates were removed and wells containing hatching eggs were transferred to plastic infection chambers (250 ml, 9 cm diameter, 5 cm depth) filled with FSW and fitted with a $105 \mu\text{m}$ nylon mesh lid. Kearn et al. (1992a) demonstrated that *Benedenia seriolae* eggs hatch during daylight, so infection chambers were placed in the 2 temperature controlled tanks containing experimental unparasitised fish during the hours 06:00 to 12:00 h, at which time water flow to tanks was suspended. Infection chambers prevented escape of eggs into tanks while allowing hatched larvae to swim freely into the tank containing unparasitised fish. After the 6 h infection period, chambers were removed from the tank and their contents preserved in 1% formalin. Egg wells were examined for empty eggs using a stereomicroscope to ensure that hatching had occurred.

Due to the expected influence of water temperature on parasite growth, different numbers of fish were required at each temperature: 43 fish at 14°C , 41 fish at 18°C , 35 fish at 22°C and 30 fish at 26°C . Experiments at 14°C and 18°C were run concurrently with sampled fish returned to a separate, parasite-free holding tank. At the conclusion of the 14°C experiment, the tank temperature was raised to 22°C and previously sampled fish were cleaned again and experimentally re-

infected in this tank, as described above. The 26°C experiment commenced at the conclusion of the 18°C experiment, using the same fish population.

Effect of water temperature on parasite growth. At each temperature, fish were sampled and parasites removed at regular intervals post infection (p.i.): 1 fish was sampled every 3 d at 14 and 18°C, every 2 d at 22°C and daily at 26°C. Sampling began 1 d p.i. Fish were anaesthetised in separate 60 l tanks (600 × 360 × 260 mm) containing 0.6 ml clove oil in 30 l of seawater filtered through a 75 µm mesh, then transferred by hand to a second 60 l tank containing 30 l of dechlorinated tap water for 5 min to kill *Benedenia seriolae* infecting fish (Chambers & Ernst 2005). Any parasites remaining attached to fish were removed by gently rubbing fish skin surfaces by hand. Older (i.e. larger) worms were gently removed using fine forceps. Water from the anaesthetic and tap water bathing tanks was filtered using a 75 µm mesh filter, back-flushed into a 250 ml sample jar, and the contents were preserved in 1% formalin. Low formalin concentrations were sufficient to preserve parasites owing to the low tissue/fixative ratio. Parasite numbers per sampled fish were counted within 24 h and all specimens were stained with Mayer's haematoxylin, dehydrated in an ethanol series, cleared in cedar wood oil and mounted in Canada balsam. Parasite total length (parasite TL, including the haptor), maximum parasite width, accessory sclerite length and anterior hamulus length were measured (Fig. 1) using a computerised digitising system as described by Roff & Hopcroft (1986). Posterior hamulus length was not measured because these haptor sclerites were difficult to locate in older (larger) parasites.

Determination of age at sexual maturity. Parasite age at sexual maturity was determined at each water temperature by assessing the development of male and female organs and the presence of eggs in the female reproductive system of mounted specimens. Organs assessed were testes, male copulatory organ and vas deferens (male) and ovary, ootype, uterus, vitellarium and vitelline reservoir (female). At 28°C, *Benedenia seriolae* may reach sexual maturity at a parasite TL of approximately 4 mm (I. Ernst & I.D. Whittington unpubl. data). Therefore, when parasite TL in samples approached 3.5 mm and reproductive organs neared full development, the sampling rate was increased to 1 fish per day to determine the day on which eggs were first produced. A single fish at each temperature was randomly selected, isolated for 1 h in a 60 l tank containing 30 l of filtered, aerated seawater and sampled as above to obtain total parasite numbers, parasite morphometric and developmental data. Isolation tank water was filtered, as described previously, to determine whether *B. seriolae* eggs were present.

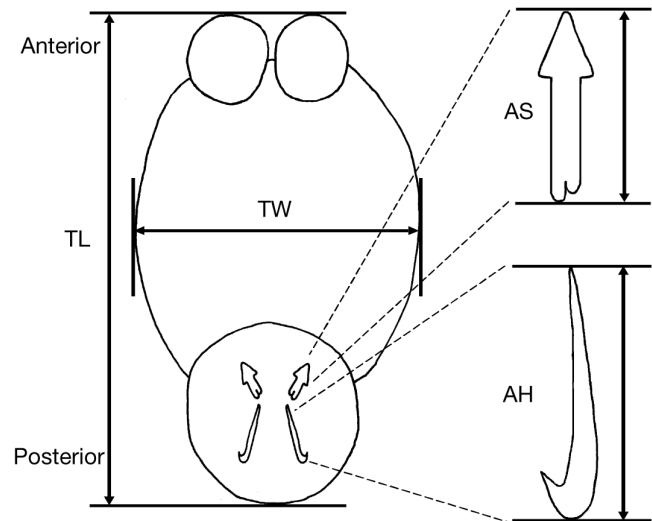


Fig. 1. *Benedenia seriolae*. Morphometric parameters: TL, parasite total length (including haptor); TW, maximum parasite width; AS, accessory sclerite length measured from distal point to longest bifid extremity; AH, anterior hamulus length. Posterior hamuli (not drawn) were not measured owing to difficulty in locating them in older (larger) specimens

Attainment of sexual maturity was confirmed by the first sign of eggs either in tank filtrate or in the female reproductive system of mounted parasites.

Analysis. The relationship between water temperature and parasite age was plotted for all 4 parasite morphometric parameters at each experimental temperature and analysed using 1-way ANOVA, and the *F*-ratio was used to determine the significance of the test.

RESULTS

Stages of development for *Benedenia seriolae*

Five developmental stages were assigned to *Benedenia seriolae* based on the successive appearance of reproductive organs (Fig. 2). Development was protandrous with male organs developing first (Fig. 2A–C) followed by female organs (Fig. 2C–E). A detailed developmental sequence based on 14 specimens per stage at 18°C, including the total length range for each developmental stage from post larva to sexually mature adult, is shown in Fig. 2. The presence of eggs either in the ootype of mounted specimens or in seawater containing isolated, experimentally infected fish (see below) confirmed the attainment of sexual maturity (Stage 5, see Fig. 2E). Regardless of water temperature (14, 18, 22 and 26 ± 0.5°C), sexual maturity of *B. seriolae* was attained at a similar mean parasite TL (3.9 mm ± SE 7.46 µm).

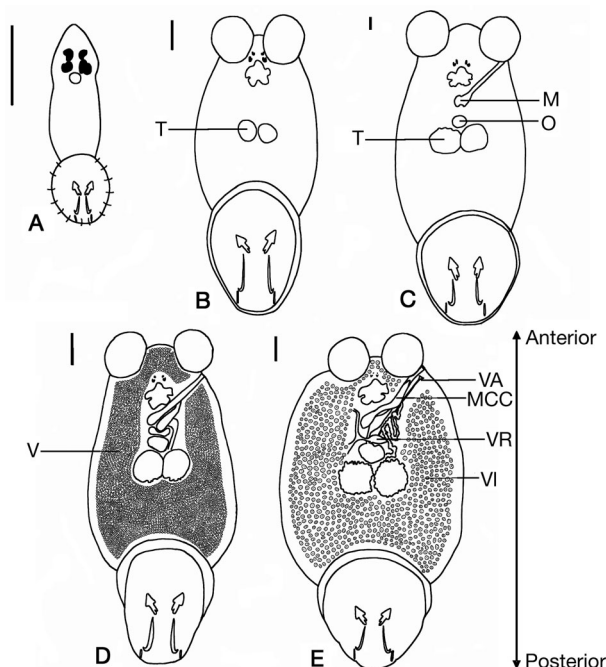


Fig. 2. *Benedenia seriolae*. Successive developmental stages based on 14 specimens per stage at $18 \pm 0.5^\circ\text{C}$. (A) Undifferentiated post larva; no reproductive organs (TL: 0.26–1.0 mm). (B) Appearance of testes (T); no female reproductive organs (TL: 1.1–2.1 mm). (C) Further development of testes, male copulatory organ (M) and appearance of ovary (O) (TL: 2.2–2.8 mm). (D) Male reproductive system (testes, male copulatory organ, vas deferens) fully developed; further ovarian development; appearance of compact vitelline follicles (V) (TL: 2.9–3.8 mm). (E) Sexually mature specimen with functional vitellarium able to lay eggs; male copulatory complex (MCC), vagina (VA), vitellarium (VI) and vitelline reservoir (VR) (TL: > 3.9 mm). Regardless of water temperature ($14, 18, 22$ and $26 \pm 0.5^\circ\text{C}$), sexual maturity was attained at a similar mean parasite TL ($3.9 \text{ mm} \pm \text{SE } 7.46 \mu\text{m}$) and a similar mean anterior hamulus length ($292.5 \pm \text{SE } 0.236 \mu\text{m}$). Scale bars (A–C) = $100 \mu\text{m}$; (D–E) = $50 \mu\text{m}$

Effect of water temperature on parasite growth rates

Experiments continued for the following durations: 76, 45, 26 and 24 d p.i. at $14, 18, 22$ and $26 \pm 0.5^\circ\text{C}$, respectively. Time to attain sexual maturity was determined from a range of 4 to 17 *Benedenia seriolae* specimens removed from single isolated fish specimens at each water temperature, when freely deposited eggs were first detected in isolation tank filtrate or eggs were observed in the female reproductive system in mounted parasites. Parasite age at the first sign of sexual maturity was strongly influenced by water temperature and was attained at 41, 24, 16 and 14 d p.i. at $14, 18, 22$ and $26 \pm 0.5^\circ\text{C}$, respectively (Fig. 3). Means of parasite TL, maximum parasite width, accessory sclerite length and anterior hamulus length from parasite samples were used to assess parasite growth

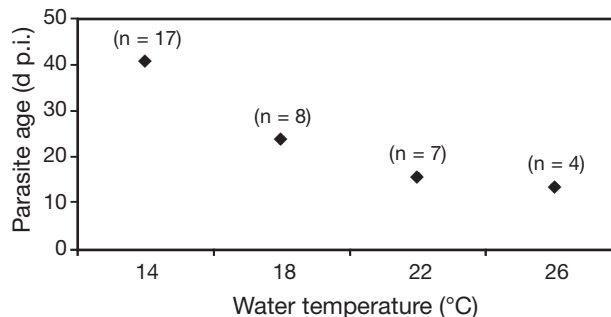


Fig. 3. *Benedenia seriolae*. Relationship between age (d p.i.) at sexual maturity (onset of egg production) and water temperatures between 14 and $26 \pm 0.5^\circ\text{C}$, determined from parasite populations of single fish specimens isolated to assess the first sign of parasite eggs in tank filtrate or in the female reproductive system of mounted specimens; n, no. of parasites recovered from experimental fish at each water temperature

at different times p.i. at each water temperature (Fig. 4A–D, respectively). All parameters measured indicate that parasite growth was continuous for the duration of the experiments, with steeper slopes indicating faster growth at higher water temperatures (Fig. 4). Plots of mean parasite TL, mean maximum parasite width and mean accessory sclerite length against parasite age were statistically linear (Fig. 4A–C, respectively, $p < 0.001$); however, the plot of mean anterior hamulus length versus parasite age (Fig. 4D) demonstrated the best fit to a linear relationship at each temperature (as determined by R^2 values). Therefore, anterior hamulus length ($R^2 = 0.98$) displayed near-constant growth rates at each experimental temperature. Of the parameters measured, anterior hamulus length was considered the most reliable index of parasite age, not only because of a near-constant growth rate, but also because of the relative ease with which these large haptor sclerites can be identified and measured. Furthermore, the anterior hamuli comprise hard sclerotised protein, which, unlike soft body tissues, are affected less by fixation and preparation methods.

Application of these data

After establishing experimentally that mean anterior hamulus length in *Benedenia seriolae* is a useful and reliable indicator of parasite age, data were inverted to generate an anterior hamulus growth index (AHGI) in terms of time per unit length ($\text{d } \mu\text{m}^{-1}$; Fig. 5). This is a significant relationship ($R^2 = 0.9999$; F -ratio from 1-way ANOVA = 591866.99) and the curve in Fig. 5 is described by the following quadratic equation (where T = water temperature):

$$\text{AHGI (d } \mu\text{m}^{-1}) = 0.500263 - 0.0367877 T + 0.00074666 T^2 \quad (1)$$

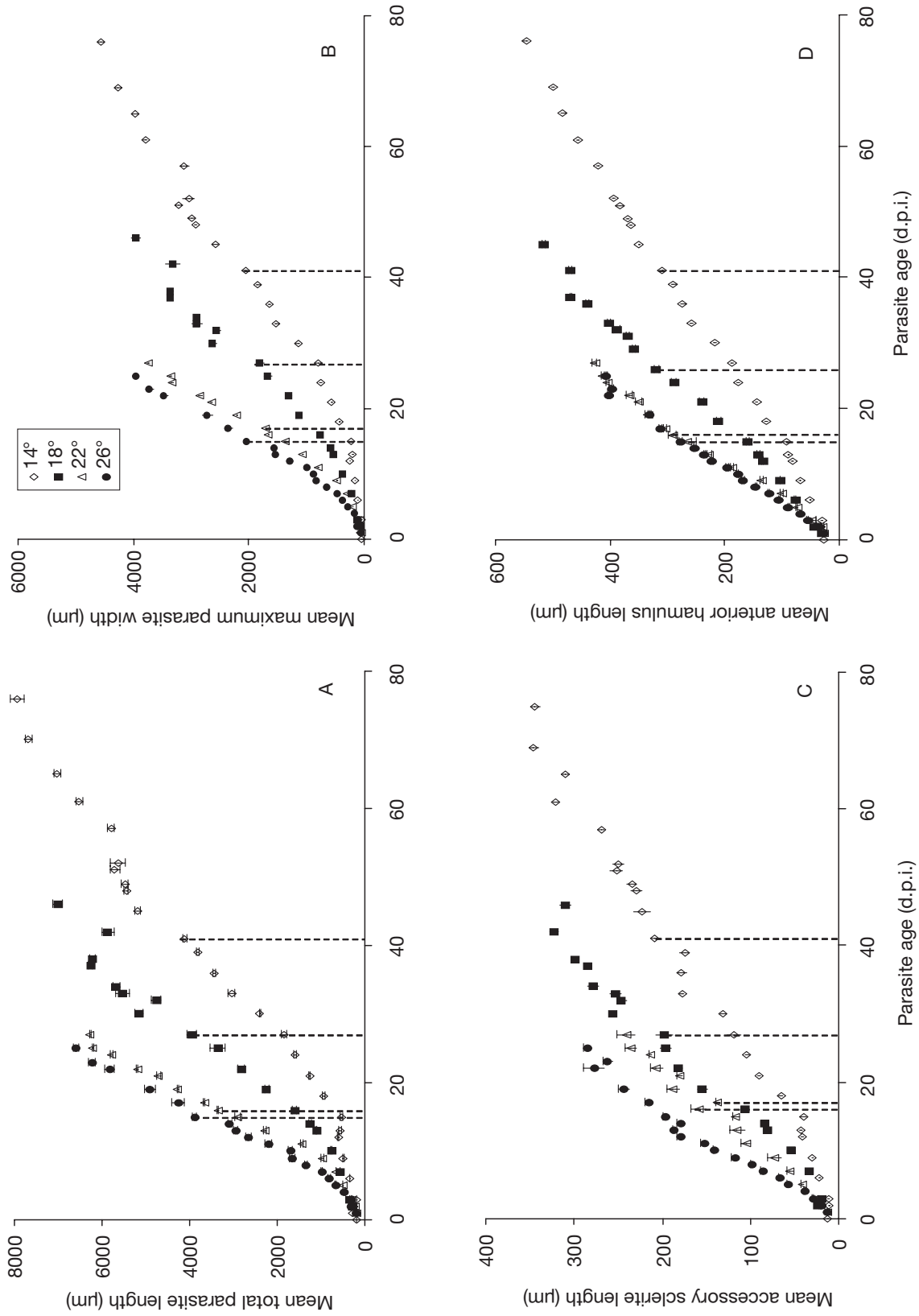


Fig. 4. *Benedenia seriolae*. Relationship between different water temperatures and (A) mean parasite TL ($R^2 = 0.95$), (B) mean maximum parasite width ($R^2 = 0.91$), (C) mean accessory sclerite length ($R^2 = 0.97$) and (D) mean anterior hamulus length ($R^2 = 0.98$) at different ages (d p.i.). Mean measurements presented in $\mu\text{m} \pm \text{SE}$. Vertical dashed lines indicate mean values for each parameter at sexual maturity at each water temperature

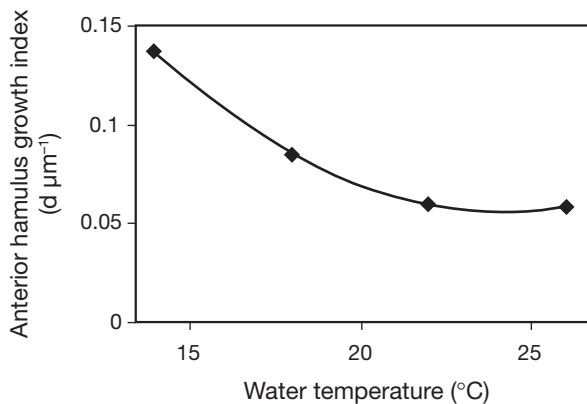


Fig. 5. *Benedenia seriolae*. Mean anterior hamulus growth index (AHGI) ($\text{d } \mu\text{m}^{-1}$) for water temperatures between 14 and $26 \pm 0.5^\circ\text{C}$, derived from inverting mean anterior hamulus length at sexual maturity for each water temperature (T). Equation of Line: $\text{AHGI} (\text{d } \mu\text{m}^{-1}) = 0.500263 - 0.0367877T + 0.00074666T^2$ (1-way ANOVA, F -ratio = 591866.99; $R^2 = 0.9999$)

This relationship allows current parasite age to be determined using information about mean anterior hamulus length at a known water temperature between 14 and 26°C . Therefore:

$$\begin{aligned} \text{current parasite age (d p.i.)} = \\ \text{mean anterior hamulus length} \times \text{AHGI} \end{aligned} \quad (2)$$

Mean anterior hamulus length at sexual maturity at all water temperatures was $292.5 \pm \text{SE } 0.236 \mu\text{m}$ and is termed the 'anterior hamulus length at sexual maturity constant'. Applying these data for water temperatures between 14 and 26°C :

$$\begin{aligned} \text{parasite age at sexual maturity (d p.i.)} = \\ 292.5 \mu\text{m} \times \text{AHGI (at } T \text{ of interest)} \end{aligned} \quad (3)$$

These equations can be used to calculate current parasite age for *Benedenia seriolae* on a population of captive *Seriola lalandi* and to estimate parasite age at sexual maturity at the temperature at which fish are being farmed. The benefits and application of these data are discussed below.

DISCUSSION

Benedenia seriolae is a serious problem in *Seriola* spp. aquaculture in Japan (Hoshina 1968, Egusa 1983, Ogawa & Yokoyama 1998) owing to its direct effects on fish health and indirect effects on farm efficiency. The parasite is equally problematic for the emerging *Seriola lalandi* industry in South Australia (Ernst et al. 2002) but, despite its commercial impact, there is limited knowledge of the parameters of the *B. seriolae* life cycle. A thorough understanding of these life cycle

parameters and how they are affected by environmental variables (e.g. temperature) is required for effective parasite management. Temperature is known to be a key variable effecting the life cycle parameters of capsalid parasites (Ernst & Whittington 1996, Ernst et al. 2005, Tubbs et al. 2005) and these effects must be understood if management strategies that effectively interrupt parasite life cycles are to be developed.

Previous studies of relevant capsalid biology have focused on close relatives of *Benedenia seriolae*, such as *Entobdella soleae* (see Kearns 1990), *Neobenedenia girellae* (see Bondad-Reantaso et al. 1995) and *Benedenia hoshinai* (see Ogawa 1984). Comparisons are often made among these species owing to broad similarities in morphology, systematic position and assumed similarities in parasite biology. Previous studies of life cycle parameters of *B. seriolae* have examined specimens of *Seriola quinqueradiata* from Japan (Kearns et al. 1992b) and *S. lalandi* from New Zealand (Tubbs et al. 2005). The present study describes life cycle parameters of *B. seriolae* from *S. lalandi* at water temperatures and salinity experienced by parasites and hosts in sea-cage farms in Spencer Gulf, South Australia. Data were generated on the effect of water temperature on parasite growth rate, the development of reproductive organs, age at sexual maturity and the most suitable morphometric feature for estimating parasite age. The data were used to develop criteria for determining sexual maturity of *B. seriolae* and to develop a growth index for estimating parasite age. Our findings will assist *S. lalandi* farmers to evaluate the population parameters (e.g. current parasite age, estimated age at maturity) of parasites infecting farmed fish populations, and permit refinement of whole-farm parasite management strategies.

As reported by Kearns et al. (1992b) for *Benedenia seriolae* from *Seriola quinqueradiata* in Japan and by Tubbs et al. (2005) for *B. seriolae* from *S. lalandi* in New Zealand, we found that the reproductive development of *B. seriolae* from *S. lalandi* is protandrous (Fig. 2). Several studies have confirmed protandry among the Capsalidae, including those of Ogawa (1984) on *B. hoshinai* and Whittington & Ernst (2002) on *B. lutjani*. The 5 stages reported here can be used to describe parasite development. Sexual maturity was determined based on the first appearance of a functional vitellarium, the presence of eggs in the female reproductive system and/or detection of laid eggs in tank filtrate. Accurate determination of sexual maturity is especially important for parasite management on commercial farms because effective parasite control requires knowledge of the age at which reinfesting parasites (following previous treatment of fish) first begin to lay eggs. This knowledge allows scheduling of treatments to break the parasite life cycle by killing

developing parasites before they can mature and lay eggs (which cannot be controlled or treated). We found that age at sexual maturity for *B. seriolae* is temperature dependent (Fig. 3).

The growth rates of all measured morphometric parameters (mean parasite TL, mean maximum parasite width, mean accessory sclerite length and mean anterior hamulus length) were temperature dependent. Parasites grew faster at higher temperatures, with continual growth of all parameters observed throughout experiments at each water temperature. We found that parasite TL and maximum parasite width may not be a reliable indicator of parasite age owing to variability resulting from specimen processing techniques, such as compression that can affect linear measurements of soft body tissues. Other, more reliable parameters include accessory sclerite length and anterior hamulus length because these hard sclerotised structures are less affected by fixation methods. Mean anterior hamulus length showed the best fit to a linear relationship with increasing parasite age (Fig. 4). These sclerites can be measured *in situ* with ease (Figs. 1 & 2) and therefore are the best parameter by which to estimate the age of *Benedenia seriolae*.

Kearn (1990) determined that a logarithmic relationship existed between anterior hamulus length and parasite age in *Entobdella soleae*, and concluded that continual and logarithmic growth throughout its life meant anterior hamulus length was the best index of parasite age. This was confirmed by Ogawa (1984) and by Whittington & Ernst (2002) for the capsalids *Benedenia hoshinai* and *B. lutjani*, respectively. Kearn's (1990) experiment ran over a far longer period, 185 d p.i. at 12°C, than the studies of Ogawa (1984) and Whittington & Ernst (2002), and included the life span of *E. soleae*. Our results confirm that mean anterior hamulus length is also the most reliable parameter for estimating the age of *B. seriolae*. It was beyond the scope of this study to determine the longevity of *B. seriolae*; however, the oldest parasites recovered were 76 d p.i. at 14°C, and at this age, growth remained linear for all morphometric parameters (Fig. 4).

Using information on the parasite growth relationships presented in this study, parasite age and the age at which parasites will become sexually mature (for a given water temperature) can be estimated. To estimate parasite age: (1) use Eq. 1 to determine the AHGI for *Benedenia seriolae* at a given water temperature and (2) enter AHGI (as calculated above) and mean anterior hamulus length for the specimens under study into Eq. 2. If the oldest sampled parasites are juveniles, the age at which they will become sexually mature (commence laying eggs) can be estimated by entering the AHGI for the given water temperature into Eq. 3. By determining current parasite age and the predicted

age at which parasites will mature, parasite treatment can be timed to occur before developing parasites mature and begin to lay eggs. The data used to develop these equations were derived from parasite populations on *Seriola lalandi* from the Spencer Gulf and with water quality conditions experienced in the area. Although these equations may be applicable elsewhere, differing conditions (e.g. Spencer Gulf is more saline than oceanic seawater) and different host fish species may have to be considered when applying the equations presented here to different regions and host species.

In South Australia, *Benedenia seriolae* infections on farmed *Seriola lalandi* are controlled by bath treatments using hydrogen peroxide (Chambers & Ernst 2005). These treatments are highly effective at killing the juvenile and adult worms that parasitise sea-caged *S. lalandi*; however, reinfection may occur rapidly after treatment by larvae already in the water column as well as by larvae that continue to hatch from embryonating eggs in the environment near sea-cages. To control reinfection and interrupt the parasite's life cycle, treatments should be coordinated spatially and temporally. Chambers & Ernst (2005) discussed the spatial coordination required for managing *B. seriolae* on farmed *S. lalandi* in the Spencer Gulf. In the current study we presented data that will permit improved temporal coordination of treatments. Egg embryonation times for *B. seriolae* have been studied at many water temperatures and from 2 host species (see Hoshina 1968, Ernst et al. 2005, Tubbs et al. 2005). This information is necessary to determine when reinfection from embryonating eggs in the environment will occur following an initial round of spatially coordinated treatments. Subsequent treatments should be coordinated temporally to occur after all parasite eggs in the area have hatched but before the first re-infecting parasites reach sexual maturity. Our data will assist this temporal coordination by allowing current parasite age and age at maturity to be estimated at different environmental temperatures.

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