Early life-history, settlement dynamics and growth of the temperate wrasse, Notolabrus fucicola *(Richardson 1840), on the east coast of Tasmania.*

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Declarations

I hereby declare that this thesis is my own work, except where due acknowledgement is given, and that the material presented here has not been submitted at another university for the award of any other degree or diploma.

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Dirk Cameron Welsford

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Table of Contents

Figures and Tables

CHAPTER 2

CHAPTER 3

CHAPTER 4

CHAPTER 5

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Abstract

Temperate reef fishes in general have received less attention than their tropical counterparts, despite their prominence in shallow reef ecosystems and developing fisheries. The temperate wrasses are a case in point, where a commercial fishery in southeastern Australia has developed over the last decade, targeting the large species in the genus *Notolabrus*. More research directed at the biology and ecology of temperate wrasses is required to improve the understanding of wrasse population dynamics and inform the management of fisheries targeting these species. This thesis describes original studies investigating the biology and ecology of all life-history stages of *Notolabrus* spp. in Tasmania.

The development of *Notolabrus* spp. is described from eggs to post-settlement juveniles, based on reared larvae and wild caught pre- and post-settlement specimens. The early life stages of the two species of Tasmanian *Notolabrus* are morphologically very similar and cannot be readily identified by traditional methods, such as meristic or pigmentation pattern analyses. A random fragment length polymorphism (RFLP) assay is developed to discriminate all the 5 Tasmanian species in the pseudolabrine group (*Notolabrus* and closely related genera).

Ageing fish using otolith microstructure requires species-specific validation of the onset and periodicity of increments. The presence of daily increments in the otoliths of *N. fucicola* and *N. tetricus* is validated using oxytetracycline (OTC). The initiation of daily increments is determined, based on comparisons of the sagittal otoliths of reared larvae and the primordial region in wild caught post-settlement specimens. Daily increments are initiated in the larval otolith at yolk sac absorption, which may occur up to 14 days after fertilisation at 10 °C.

Otolith microstructure is used to determine spawning and settlement dates of *N. fucicola* and *N. tetricus* at three sites on the east coast of Tasmania, over three years. The planktonic larval duration of *N. fucicola* varies from 40 – 87 days, back-calculated from the settlement check observed in sagittal otoliths. The settlement season for these species extends for more than 100 days over spring and summer, a period of high variability in the oceanographic conditions off the Tasmanian coast. Patterns of sea surface temperature (SST) are correlated with peaks of spawning dates, larval duration, and post-settlement growth. Settlers show earlier spawning dates in years with higher SSTs. Mean larval duration decreases as SST increases in each year. Growth is significantly faster in later settlers within a year class. A trade-off is apparent between earlier settlers spending longer in the risky planktonic phase, but arriving on the reef earlier and hence having longer to grow in the post-settlement habitat before winter, as against later settlers' shorter exposure to the planktonic environment but shorter growth phase post-settlement before water temperatures and productivity plunge.

The growth of demersal juvenile and adult *N. fucicola* is modelled using otolith and tag- recapture data. Robust methods of comparing growth models are developed, involving likelihood ratio tests, and percentile confidence intervals and 2D plots of parameters estimates derived using bootstrapping. Traditional models for describing growth in fishes, such as the von Bertalanffy growth function show high levels of correlation in their parameter estimates, and are best compared using likelihood ratio tests, and inspection of parameter plots. *Notolabrus fucicola* growth varies seasonally, between sites, and at one site growth difference between sexes is detected. Such growth variability poses a challenge to management based on models assuming uniform growth. Growth variability in *N. fucicola* may explain why the

species is a gonochorist, rather than a protogynous hermaphrodite as in most other wrasses species. Sex change may have been abandoned because attaining and/or maintaining a clear size advantage over other members of a social group to control mating opportunities may be impossible under unpredictable growth conditions.

1. Introduction: The ecology of temperate wrasses

Wrasse diversity

Fishes of the family Labridae, the wrasses, are one the most speciose and widespread fish families, and with nearly 500 species described, they are third in number only to the Gobiidae and the Serranidae (Watson 1996; Parenti and Randall 2000). They have representatives extending from the high (40°+ S) latitudes of the southern hemisphere of Tasmania and New Zealand, throughout the tropics and as far north as the sub-arctic waters of Finland and the north-eastern Atlantic, extending to the limits of perciform fish distribution (Choat and Bellwood 1991).

Although they are morphologically relatively conservative, with some genera being nearly identical meristically (Russell 1988; Gomon *et al.* 1994; Watson 1996), they show a wide variety of form and size, ranging from tiny species, reaching maximum sizes of only 8.5 cm standard length (e.g. *Dotalabrus alleni*, Gomon *et al.* 1994), to giants of 3 m total length (e.g. *Cheilinus undulatus*, Choat and Bellwood 1991; Watson 1996). Ecologically, they are found commonly as members of shallow water fish faunas, over coral reefs in the tropics and in rocky and kelp covered reefs in the temperate waters, where they feed on benthic invertebrates, particularly crustaceans and molluscs (Jones 1988; Choat and Bellwood 1991; Watson 1996; Denny and Schiel 2001; Shepherd and Clarkson 2001).

Complex suites of social behaviours accompany the diverse reproductive biology of wrasses, with species representing a spectrum of strategies from gonochorism through to protogynous hermaphroditism, and with both initial phase males and terminal phase males (Thresher 1984). Depending on the species, spawning

may take place every day, all year round, as in the coral reef species *Thalassoma bifasciatum* (Robertson *et al.* 1999), or be restricted to a circumscribed season as in the north-eastern Atlantic species *Tautoga onitis* (Hostetter and Munroe 1993). Mating may involve pairs of the opposite sex, or groups of males, including initial phase 'sneaker' males attempting to fertilise a female's eggs. The majority of wrasses spawn small, pelagic eggs, but there are also examples in northern European species such as *Ctenolabrus exoletus*, *Symphodus melops, Labrus bergylta* and *L. mixtus* that build nests, lay benthic eggs, and care for the young up to hatching (Darwall *et al.* 1992).

Life-history strategies

As a result of their ubiquity and diversity, and the relative ease of observation in warm, clear, shallow waters, tropical wrasses have become a test bed for many hypotheses regarding the evolution of life-history strategies, such as the relationship between sex change schedules and population density (reviewed by Warner 1991), and the relationships between early life-history, recruitment and adult population dynamics (Victor 1982; Victor 1986a; Victor 1986b; Victor 1986c; Victor 1987; Robertson *et al.* 1988; Wellington and Victor 1989; Kishiro and Nakazono 1991; Wellington and Victor 1992; Hare *et al.* 1994; Sponaugle and Cowen 1997; Robertson *et al.* 1999).

Although they are often as common and obvious in temperate reef ecosystems as are their tropical counterparts, the life-history strategies of temperate wrasses have received comparatively little attention until recently. Moreover, the majority of work on temperate species, including detail on aspects of early life-history and population dynamics, relates to northern hemisphere species. Northern American species studied

include the only two wrasses found on the north Atlantic coast, *Tautoga onitis* (Sogard *et al.* 1992; Hostetter and Munroe 1993; Arendt *et al.* 2001) and *Tautoglabrus adspersus* (Levin 1993; Levin 1994; Levin 1996; Levin *et al.* 1997; Nitschke *et al.* 2002), and *Semicossyphus pulcher* on the Californian coast (Cowen 1985; 1990; 1991). Life histories of northern European wrasses have also been reviewed, precipitated by their commercial use as cleaner fish in salmon aquaculture (Darwall *et al.* 1992), and studies of warm temperate wrasses in Japan include detailed descriptions of embryonic and larval development (Mito 1962; Okiyama 1988; Kimura *et al.* 1998), reproductive biology and behaviour (Shigeta *et al.* 1994; Matsumoto *et al.* 1997). This emphasis on northern temperate species has developed despite that, relative to the northern hemisphere, the diversity of temperate wrasses around Australia and New Zealand is much greater. Around the Tasmanian coastline alone, at least eight different species in seven genera of labrids are reported, six species of which are commonly found on the east coast (Russell 1988; Gomon *et al.* 1994; Edgar 1997). This contrasts with northern Europe, where only 5 species occur over this vast area (Darwall *et al.* 1992).

An notable exception to this trend is the work of Jones in the early 1980s on patterns of recruitment, population dynamics and reproductive behaviour of *Notolabrus celidotus* in northern New Zealand (Jones 1980; Jones and Thompson 1980; Jones 1983; Jones 1984a; Jones 1984b), which occurred in parallel with a period where research in the tropics had a veritable hegemony on 'reef fishes'. The biology of southern hemisphere temperate species thereafter received little coverage until Barrett described aspects of the reproductive biology and home-ranging behaviour of *N. fucicola* and *N. tetricus*, *Pictilabrus laticlavius* and *Pseudolabrus psittaculus* (Barrett 1995b), and spatial variability in growth of *N. fucicola* and *N.*

tetricus (Barrett 1999) on the reefs of southeastern Tasmania. These studies, alongside recent work on the trophic ecology of *N. tetricus* in south Australia (Shepherd and Clarkson 2001), reproduction and diet of *N. fucicola* on New Zealand reefs (Denny and Schiel 2001; Denny and Schiel 2002), and reproduction, growth and movement of the large protogynous warm temperate wrasse, *Achoerodus viridis* (Gillanders 1995; Gillanders and Kingsford 1996; Gillanders 1997; Gillanders and Kingsford 1998), constitute a resurgence in studies directed at temperate representatives of the labridae.

Biology and ecology of Notolabrus

The totality of studies on southern temperate wrasses reveals that the biology and ecology of temperate wrasses is as diverse and compelling as that of their tropical counterparts. Within the genus *Notolabrus* alone, a spectrum of sexual strategies exists. In the small (maximum size around 25 cm), rapidly maturing, protogynous *Notolabrus celidotus*, individuals may mature into an initial colour phase as either cryptic males or females and all terminal phase males are derived from mature females (Jones 1980). Larger females inhibit the maturation of subordinate females through aggressive encounters (Jones and Thompson 1980), and spawning sites are chosen by the females, who leave their home ranges to spawn within male territories on the deep seaward edge of reefs (Jones 1980). By contrast, the protogynous blue-throat wrasse (*N. tetricus*) is much larger and longer lived, with only terminal phase males, and sex change occurs at approximately 27-35 cm total length (Barrett 1995a). Females are home-ranging, and males maintain a territory that overlaps the home ranges of a group of females (Barrett 1995a; Barrett 1995b). The purple wrasse (*Notolabrus fucicola*) is similarly large and long lived (Barrett 1999; Ewing *et al.* in press), but is the only gonochoristic species in the genus, with no recorded examples

of sex change (Barrett 1995a; Denny and Schiel 2002). Purple wrasse maintain home ranges, and have also been observed aggregating to spawn on reef edges, with groups of males simultaneously streaking into the water column with each spawning female (N. S. Barrett, pers. comm.[1])

Wrasse fisheries

The recent interest in temperate wrasses is no doubt motivated in part by a shift towards the commercial exploitation of large temperate reef species, including wrasses, morwongs (Cheilodactylidae) and leatherjackets (Monacanthidae), and trophic interactions between large wrasses and commercially valuable invertebrates such as abalone (Dayton *et al.* 1998; Shepherd and Clarkson 2001). This is the case for *N. fucicola* and *N. tetricus* in southeastern Australia, where a market for live wrasse (and morwong) was developed in the early 1990s, mainly for Asian restaurants in Sydney and Melbourne. Catches of wrasses in Victoria total up to 60 tonnes per year, and interest in wrasse fishing is increasing in South Australia. In Tasmania, the fishery for wrasses rose from incidental catches for rock lobster (*Jasus* spp.) bait of a few tonnes per year in the late 1980's, to a peak in catch of 1993-94 of 178 tonnes, with an estimated landed value of A$ 0.5 million (Lyle and Hodgson 2001). This parallels the development in California of a live fishery for *Semicossyphus pulcher*, which rose from 16 tonnes to 195 tonnes between 1989 and 1995, having drastic effects on the *Macrocystis* forest ecosystem on the eastern Pacific coast (Dayton *et al.* 1998).

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[1] Dr Neville S. Barrett, Research Scientist. Taroona Marine Research Laboratories, Tasmanian Aquaculture and Fisheries Institute, University of Tasmania.

Commercial fishers in Tasmania do not routinely distinguish wrasse catches by species, although *Notolabrus fucicola* and *N. tetricus* are known to be targeted by trap and hook fishing. In Victoria and South Australia, *N. tetricus* is the dominant wrasse species targeted by commercial fishers. In Tasmania and Victoria, management involves limits on effort involving a fixed number of live fish specific endorsements, limits of two traps per operator (in Tasmania), and size limits. To sustain this fishery successfully, information on the life-history and population dynamics of targeted wrasse species are needed, including description of recruitment processes and estimates of growth. This will facilitate the development of models for stock assessment and assist scientifically-based management,

Thesis outline

As a precondition for the development of broad theories concerning reef-fish population dynamics the evolution of reef-fish life-history strategies, empirical studies to provide the specific detail of fishes' ecology and biology must be conducted.

Several significant omissions exist in the volume of work on southern temperate wrasses. No study has dealt in any detail with the early life-history up to and including settlement. This is in part due to the great difficulty of observing, sampling and identifying the early life stages of wrasses. Their larvae in general show few external identifying characteristics, with none of the head spination, elaborate morphological adaptations or pigmentation that are routinely used in the identification of other fish taxa (Leis and Rennis 1983; Richards and Leis 1984; Neira *et al.* 1998). Descriptions of the eggs and larvae of in the pseudolabrine group (wrasses in the genus *Pseudolabrus* and allied genera, including *Notolabrus*, *sensu* Russell 1988) are sparse, and based exclusively on New Zealand *N. fucicola* and *N. celidotus* (Elder

1966; Robertson 1973; Robertson 1975; Crossland 1981), or Japanese *Pseudolabrus* spp. (Mito 1962; Okiyama 1988; Kimura *et al.* 1998). In Chapter 2, I describe the early development of eggs and yolk sac larvae of *N. fucicola* and *N. tetricus* reared in the laboratory. I then develop means to identify wild caught larvae and recently settled wrasses captured on the eastern coast of Tasmania, using a combination of morphology, pigmentation characters, and a genetic assay based on mitochondrial DNA restriction fragment length polymorphisms (mtDNA RFLPs). A developmental series from egg through to post-settlement for *Notolabrus* spp. is described.

One of the most powerful tools available for the study of population and growth dynamics of fishes is the presence of regular incremental structures in their otoliths. Annual rings in otoliths have been used routinely to provide age estimates for adult fishes, but the discovery of daily increments in otolith microstructure (Brothers *et al.* 1976), which are particularly clear in young fishes, has led to accurate estimates of the timing and duration of many processes in the early life of fishes, including hatching and settlement, time spent in the plankton , and measurement of growth pre- and post- settlement (Campana and Neilson 1985; Jones 1986; Thresher 1990; Campana and Moksness 1991; Victor 1991; Campana and Jones 1992). The microstructure of wrasse otoliths has been utilised widely to determine early life-history characteristics such as settlement patterns and larval durations, once initial baseline studies validated settlement checks (Victor 1982; Wilson and McCormick 1999), and the daily nature of increments (Victor 1982; Sogard *et al.* 1992). The presence of daily increments in the otoliths of juvenile fishes is assumed in some studies. However this is a dubious practice, as examples exist where under circumstances of slow growth (e.g. Secor and Dean 1989), or cold water (e.g. Casas 1998), observable increments may form less frequently than every day, while in some

tropical and fast growing species, more than one increment per day may be observed ('sub-daily' increments e.g. Victor 1986b; Prince *et al.* 1991). Furthermore, although the timing of initial formation of daily increments can vary between species, all authors to date assume that wrasses commence forming daily increments at two days post-spawning (after Victor 1982). In Chapter 3 I describes otolith microstructure of Tasmanian *Notolabrus* larvae reared in the laboratory, in an attempt to identify when daily increments are initiated in these species. I also validate the formation of daily increments in post-settlement *N. fucicola* and *N. tetricus*, through exposure to oxytetracycline (OTC) whilst in captivity.

Using the techniques of identification and otolith microstructure analyses developed in chapters 2 and 3, the settlement dynamics of *Notolabrus* collected from artificial habitats was investigated. This included the validation of a settlement check in the otoliths and the first estimation of spatio-temporal patterns of planktonic larval duration, timing of spawning and settlement, and pre- and post-settlement growth. These results are detailed in chapter 4.

A consequence of description of the otolith microstructure of juvenile *Notolabrus* is the capacity to validate the position of the first annual ring in the otoliths of post-settlement juveniles, and therefore develop an accurate ageing method based on thin sections of sagittal otoliths (Ewing *et al.* in press). In previous studies, the growth of *Notolabrus* used whole otoliths (Shepherd and Hobbs 1985; Barrett 1999), simple tag-recapture based models (Barrett 1995a), or scales (Jones 1980). However, these studies were limited by small sample sizes or lack of validation of age estimates. Consequently, growth and growth variability in *Notolabrus* has still to be adequately described. In Chapter 5 I utilise a variety of models for describing growth in *N. fucicola*, using data derived from tag-recaptures and otolith-based age estimates

for fish from the same two populations. I also assess the suitability of statistics for comparing the parameters estimated by standard and reparameterised growth models, using bootstrapping and likelihood ratio tests.

In Chapter 6, the general discussion, I synthesise the results, and discuss ideas as to the most productive ways forward for future research. These include opportunities for continuing studies of settlement on the east coast of Tasmania, determining the evolutionary history of wrasse species in the pseudolabrine group, and the interactions between *Notolabrus* population dynamics, their life-history strategies, environmental variability and fishing pressure.

2. A description of the early life-history stages of *Notolabrus fucicola* and *N. tetricus*, and other Tasmanian labrids.

In review as Welsford, D.C, Jordan, A.R. and Smolenski, A.J. A description of the early life-history stages of *Notolabrus fucicola* and *N. tetricus*, and other Tasmanian labrids. New *Zealand Journal of Marine and Freshwater Research*

Abstract. The developmental stages of *Notolabrus* spp. from egg to post-settlement juvenile are described and illustrated, including notes on other Tasmanian labrid species. Developmental stages examined included eggs and yolk sac larvae reared in aquaria, larvae from ichthyoplankton samples, and newly settled juveniles collected from artificial habitats, collected from the coastal waters of eastern Tasmania. Development and morphology were similar to other labrids previously described. Species-specific characters were only evident after settlement, and with the development of scales. A molecular assay, based on restriction fragment length polymorphism (RFLP), was successfully applied enabling the discrimination of all 5 pseudolabrine wrasses found on the east coast of Tasmania. The results of this study constitute the first description of the early life stages of pseudolabrine wrasses from temperate Australia.

Introduction

The Labridae (wrasses) is the third most speciose fish family, after the Gobiidae and the Serranidae, with up to 500 species occurring on temperate and tropical reefs worldwide (Watson 1996; Parenti and Randall 2000). Six species of labrids are reported from the east coast of Tasmania (Gomon *et al.* 1994; Edgar 1997). Apart from the crimson cleaner wrasse, *Suezichthys aylingi* (Russell, 1985), all belong to four genera in the pseudolabrine group (*sensu* Russell 1988), namely the purple wrasse, *Notolabrus fucicola* (Richardson, 1840), blue-throated wrasse, *N. tetricus* (Richardson, 1840), senator wrasse, *Pictilabrus laticlavius* (Richardson, 1839), Castlenau's wrasse, *Dotalabrus aurantiacus* (Castlenau, 1872) and rosy wrasse, *Pseudolabrus psittaculus* (Richardson, 1840). Like many labrid groups, pseudolabrines are meristically very conservative. Genera can be identified on the basis of *in vivo* colouration, the position of canine teeth, number of pectoral fin rays, shape of the dorsal fin membrane, the shape of scales at the base of the dorsal fins and the position of the dorsal margin of the preopercle (Russell 1988).

Notolabrus fucicola and *N. tetricus* are two of the most common reef–associated fish species that occur in Tasmania. *Notolabrus fucicola* also occurs around New Zealand, and along the coast of south-eastern Australia from southern New South Wales west to Kangaroo Island in South Australia, while *N. tetricus* is found from Sydney to the Great Australian Bight (Russell 1988; Edgar 1997). These species are morphometrically and meristically near identical, with *N. fucicola* characterised by having 2-5 rows of cheek scales, as opposed to one row in *N. tetricus* (Russell 1988; Gomon *et al.* 1994). By contrast, these species differ in their growth rates, behaviour and mating systems (Barrett 1995a; b). *Notolabrus fucicola* is a gonochorist (sex is fixed at maturity), while *N. tetricus* is a sexually dimorphic protogynous

hermaphrodite, where fish mature initially as females and may transform into males with growth and social dominance (Barrett 1995a). *Notolabrus* spp. play a significant role in the trophic dynamics of temperate reefs in their range (e.g. Shepherd and Clarkson 2001; Denny and Schiel 2001), and are increasingly economically important with the development of a live export fishery based on both species in southeastern Australia (Lyle and Hodgson 2001).

To achieve a better understanding of the population dynamics of these species, accurate identification of early life stages is needed. The characters used to distinguish adult pseudolabrine species are not suitable for distinguishing the pre-settlement stages, as wrasses do not develop teeth or scales until after settlement (Richards and Leis 1984), pectoral fin ray counts may vary and overlap between pseudolabrine genera (Gomon *et al.* 1994; Russell 1988), and juvenile colouration is often very different to adult patterns (Kuiter 1993).

Previous descriptions of the early life-stages of wrasses largely relate to tropical or northern hemisphere temperate species, with identification of larvae to species generally based on pigmentation patterns (e.g. Mito 1962; Fahey 1983; Leis and Rennis 1983; Richards and Leis 1984; Okiyama, 1988; Kimura and Kiriyama 1993; Kimura *et al.* 1998; Dulčić *et al.* 1999). Specific information relating to the early life-stages of pseudolabrine wrasses is limited to Japanese and New Zealand species. Egg and larval stages of *Pseudolabrus* spp. from Japanese waters are illustrated by Mito (1962) and Okiyama (1988). Elder (1966) illustrated post-flexion larvae (9.0-10.7 mm total length) and post-settlement juveniles (14.1-16.2 mm total length) of *N. celidotus* captured in November–January in Wellington Harbour. Crossland (1981) illustrated a single post-flexion larvae (13.2 mm total length) of *N. celidotus* captured in New Zealand coastal waters. Robertson (1973) described pelagic

eggs of *N. cēlidōtūs* (Blōch & Schneider, 1801) and *N. fucicola,* both occurring in the inshore waters of New Zealand in spring, and reared *N. fucicola* eggs, which hatched after 120 hours at 10 °C.

A comparison of post-flexion *Pseudolabrus* spp. (Okiyama 1988) and *N. celidotus* (Elder 1966; Crossland 1981) indicates some differences in pigmentation that may be species-specific. However, within genera, few external characters may distinguish early life stages in the pseudolabrines. Molecular taxonomy using a protocol based on analysis of restriction fragment length polymorphisms (RFLP) represents an efficient technique to distinguish taxonomically conservative groups of adult fishes (e.g. Chow *et al.* 1993; Aoyama *et al.* 2000; Takeyama *et al.* 2001), and to establish the identity of fish in their larval stages (e.g. Lindstrom 1999). This method was considered to establish specimen identity when other methods for identifying early life stages, such as the series method (*sensu* Neira *et al.* 1998), provide ambiguous results.

This study describes the development of *Notolabrus* spp. from eastern Tasmanian waters, using animals reared in the laboratory and captured in the wild, and illustrates the major developmental stages of larvae in this genus, from yolk sac to post-settlement juveniles. A RFLP assay is described that successfully discriminates between *N. fucicola* and *N. tetricus*, and the other pseudolabrine genera found in Tasmania.

Materials and Methods

Eggs and reared larvae

To determine the characteristics of eggs and early larvae of *N. fucicola* and *N. tetricus*, gametes were collected from adults captured on the east coast of Tasmania, eggs fertilised *in vitro* and larvae reared in aquaria under different temperature regimes. Eggs from both species were reared at a constant temperature of 11 °C, and one batch of *N. tetricus* at a constant 14.5 °C. Constant temperature rearing was conducted in 9 L aquaria with aeration and daily water changes. Two further batches of eggs of *N. fucicola* and *N. tetricus* were reared at ambient sea temperature (17.1-18.6 °C). Eggs at ambient temperature were reared in 210 L tanks with aeration from the tank bottom, and flow-through of water filtered to 50 μm at 120 $L.hr^{-1}$. No method of bacterial control, other than physically removing debris from the bases of the tanks daily was employed.

Samples of 10 recently fertilised eggs from each species were digitised and the whole egg and oil droplet diameters were measured to the nearest 0.01 mm using image analysis software. Samples of 3-10 eggs or larvae were collected at intervals during rearing and preserved, either in 4% formalin in seawater, or in absolute ethanol. Observations on morphology and pigmentation were recorded on fresh material, to counter shrinkage or distortion due to preservation.

Wild caught larvae

Based on the appearance of the most developed *Notolabrus* spp. larvae from rearing experiments, the smallest *Notolabrus* spp. post-settlement juveniles, and the general characteristics described for labrid larvae (Elder 1966; Crossland 1981; Leis and Rennis 1983; Okiyama 1988), larvae were sorted from archived samples collected

during ichthyoplankton surveys of eastern Tasmanian coastal waters, captured using oblique tows of a 500 μm bongo net and stored in absolute ethanol (Jordan *et al.* 1995).

Larval descriptions

Wild caught and reared larvae were examined under a stereomicroscope using transmitted and reflected light. The position, size and number of melanophores, and the development of fins were noted. An image of each larva was digitised and measurements (to the nearest 0.1 mm) were made of standard length (SL), head length (HL), body depth (BD), pre-anal length (PAL), and presented as percentages of SL, as defined and used as a standard suite of measurements for larval descriptions in Neira *et al.* (1998).

Individuals at representative developmental stages were selected and illustrated with the aid of a camera lucida attached to a stereomicroscope. Size ranges bracketing the major developmental stages were derived from the smallest individual that showed evidence of entering the developmental stage, and the largest individual that had yet to fully transit the stage. Size at settlement for *Notolabrus* spp. was determined from the smallest juveniles captured post-settlement on artificial habitats.

Larvae representing all developmental stages and two morphotypes identified due to their divergent pigmentation patterns were subjected to RFLP analysis.

Adult tissue

Adult *N. fucicola* and *N. tetricus* were captured using hook and line over reef on the east coast of Tasmania. Fish were anaesthetised using clove oil, and then euthanased by chilling. A 2-3 cm^3 sample of epaxial muscle tissue was removed from each fish after death and stored in absolute ethanol.

DNA extraction and PCR amplification

Crude DNA was extracted, from approximately. 50 mg of muscle tissue in the case of adult and post-settlement juveniles, or whole fish in the case of larvae, using a modified cetyltrimethylammonium bromide (CTAB) protocol (Grewe *et al.* 1993). Crude DNA was resuspended in 50 μL of distilled water, and the concentration determined using a Versafluor fluorimeter (Bio-rad). Concentrations were determined at 30-600 $ng.\mu L^{-1}$ for adults and juveniles, but at undetectable levels for larval samples. As CTAB extraction of larvae produced no DNA suitable for amplification, further extractions of larval DNA were attempted using a DNeasy tissue kit (Qiagen), following the manufacturer's protocol. Final elution volume was 100 μL.

Using a protocol adapted from Palumbi *et al.* (1991), the primer pair L-15995 (5' AAC TCT CAC CCC TAR CTC CCA AAG 3') and H-607 (5' CTA GGG YCC ATC TTA RCA TCT TCA GTG 3'), located in the Pro-L and Phe-H tRNA genes respectively and flanking the D-loop region, were used to amplify an approximately 1000 base pair (bp) fragment of mtDNA. Amplifications were performed in a Corbett Research Palm Cycler. Each 50 μL reaction volume contained 1 unit of *Taq* DNA polymerase (Biotech International Ltd), 5μL 10X buffer (670 mM Tris, 166 mM $(NH_4)_2SO_4$, 4.5 % TritonX, 2 $mg.mL^{-1}$ gelatin), 200 μM of each dNTP, 5 μg BSA, 1 μM of each primer, 1.5 mM $MgCl_2$ and 10-60 ng of DNA. Cycling parameters were 1 cycle of 95 °C for 4 min, followed by 35 amplification cycles at 95 °C for 30 s, 56 °C for 45 s, 72 °C for 1 min, followed by a final extension for 5 min at 72 °C. Amplification products were visualised by electrophoresis on 1.0 % agarose gel stained with ethidium bromide alongside a 100 bp ladder size standard (Geneworks).

RFLP analysis

Three restriction enzymes (*Hinf* I, *Dde* I and *Bsl* I, New England Biolabs) were initially trialled on 2 adults each of *N. tetricus* and *N. fucicola*, and post-settlement juveniles that could be identified as *Pseudolabrus psittaculus* (n = 4), *Dotalabrus aurantiacus* (n = 2) and *Pictilabrus laticlavius* (n = 1). Subsequently, 10 more adults of *N. tetricus* and *N. fucicola* were trialled with *Hinf* I and *Bsl* I digestions, on the strength of easily differentiated and apparently species-specific haplotypes produced by these enzymes. In this larger assay, only *Bsl* I produced species-specific haplotypes. As distinguishing the two *Notolabrus* spp. was the primary goal, this enzyme was used in subsequent digests to attempt to identify ambiguous post-settlement *Notolabrus* specimens.

Digests contained 7.5 μL PCR product, 5 units of enzyme, 2 μL of the appropriate 10X buffer, made up to 20 μL total volume. Restriction digests were performed at 55°C (*Bsl* I) or 37°C (*Hinf* I and *Dde* I) for 1-2 hours. Digests were visualised by electrophoresis on 3.0 % Amplisize (Bio-rad) agarose, alongside a 100bp ladder size standard (Geneworks), stained with ethidium bromide, and recorded on film.

Post-settlement juveniles

Juvenile wrasses that had recently settled were captured from artificial habitats on the east coast of Tasmania (see Chapter 4). Fish were euthanased using 2 $g.L^{-1}$ benzocaine in seawater, and preserved in absolute ethanol. Specimens were measured for standard length (SL) and identified to the lowest taxonomic level possible using the keys described in Russell (1988) and Gomon *et al.* (1994).

Juvenile pigment character analysis

Post-settlement juveniles that had been identified as *Notolabrus* spp., but were of indeterminate species due to the absence or undeveloped state of distinguishing adult characters, were inspected for external pigmentation under a stereomicroscope. Small juveniles were generally lightly pigmented, and presence, absence or indeterminate state (if specimens were damaged) of pigmentation characters at 8 positions (Table 2.1) were scored for 80 individuals. Any development of scales was also noted. These individuals were subsequently subjected to RFLP analysis.

Table 2.1. External pigmentation characters scored as present, absent or indeterminate in post-settlement *Notolabrus* spp. juveniles.

Code	Character description
A	1-2 large melanophores on ventral surface of abdomen
B	Many (3+) small melanophores on ventral surface of abdomen
C	1-3 large melanophores on nape
D	1-3 melanophores on caudal peduncle
E	Melanophores on the dorsal surface between bases of rays 3 and 4 of the dorsal fin
F	Melanophores near base of 4th dorsal fin spine
G	Melanophores near base of 7th dorsal fin spine
H	Light coloured pigmentation (visible under transmitted light) in dorsal fin membrane around 8th dorsal spine

To determine if any characters were exclusive to one species, or a particular size of fish, the percentage occurrence of characters in each species group, and in two size groups (<15.0 mm, ≥15.0 mm SL) was calculated. These size groups were chosen on

the basis that 15.0 mm was close to the mean SL for both species groups, and also was the approximate size after which body scales develop.

Based on the presence/absence/indeterminate state of characters, a similarity matrix for specimens was calculated using a modified simple matching coefficient. For each pair of specimens being compared, double presences or absences of a character were weighted equally as a match. An indeterminate state in either or both specimens was counted as a mismatch. Using PRIMER 5 for Windows (©2001 Primer-E Ltd), a multi dimensional scaling (MDS) plot was constructed to examine the extent of any species- and/or size-specific clustering.

Results

Eggs and reared larvae

Recently fertilised eggs of both *Notolabrus* spp. had a smooth chorion, a narrow perivitelline space, and a pale yellow oil droplet (Fig. 2.1). *Notolabrus fucicola* eggs (1.03-1.09 mm, n = 10) were larger in diameter than *N. tetricus* eggs (0.98-1.02 mm, n = 10). Oil droplet diameters were similar in both species, at 0.17-0.20 mm and 0.17-0.19 mm, for *N. fucicola* and *N. tetricus*, respectively.

Notolabrus fucicola reared at 11°C hatched 4-5 days after fertilisation, measuring 1.70-2.14 mm SL (n = 5). No *N. tetricus* survived to hatch at 11°C. *Notolabrus tetricus* reared at 14.5°C hatched 4 days after fertilisation, and were 1.93–2.07 mm SL (n = 4). Temperatures during rearing under ambient conditions ranged from 17.1-18.6 °C. Eggs of both species began to hatch at 50 hours post- fertilisation and were all hatched within 65 hours.

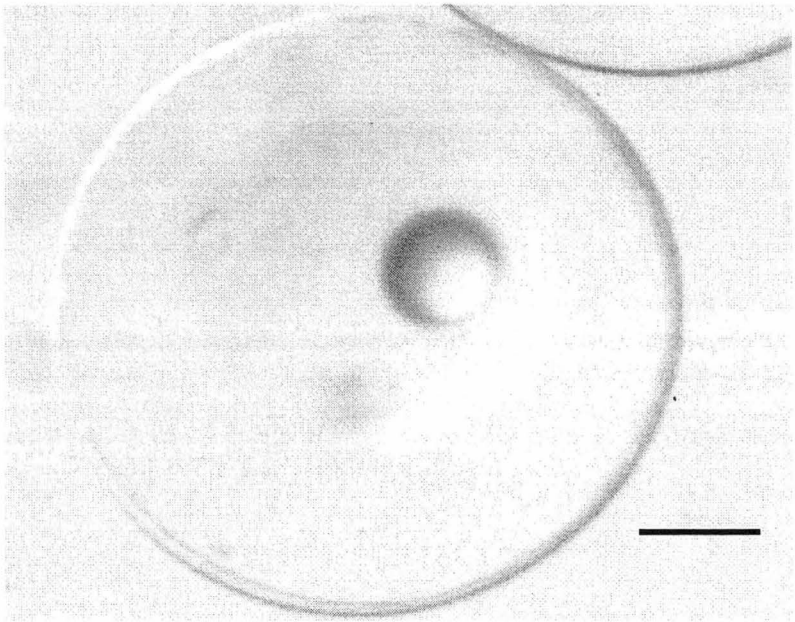

Figure 2.1. Photomicrograph of a *Notolabrus fucicola* egg prior to cleavage. Scale bar = 200 μm.

In eggs held at ambient temperature, cell division was soon evident, with eggs reaching the 16 cell stage by 200 min after fertilisation. By 24 hours myomeres were evident in the embryos. The tail bud was free by 42 hours post-fertilisation, with the embryo reaching around 75 % of the circumference of the egg. Melanophores were evident on the inside surface of the oil droplet, and scattered on the head and along the side of the body and tail (Fig. 2.2), and showed no species-specific patterns.

Newly hatched yolk-sac larvae showed no species-specific pigmentation pattern, with a few small melanophores evident on the posterior surface of the oil droplet and an irregular series of small melanophores along the dorsal surface from the nape to the tail. Small melanophores were present on the dorsal surface of the rear third of the yolk sac, and on the posterior half of the ventral surface of the tail (Fig. 2.3 a).

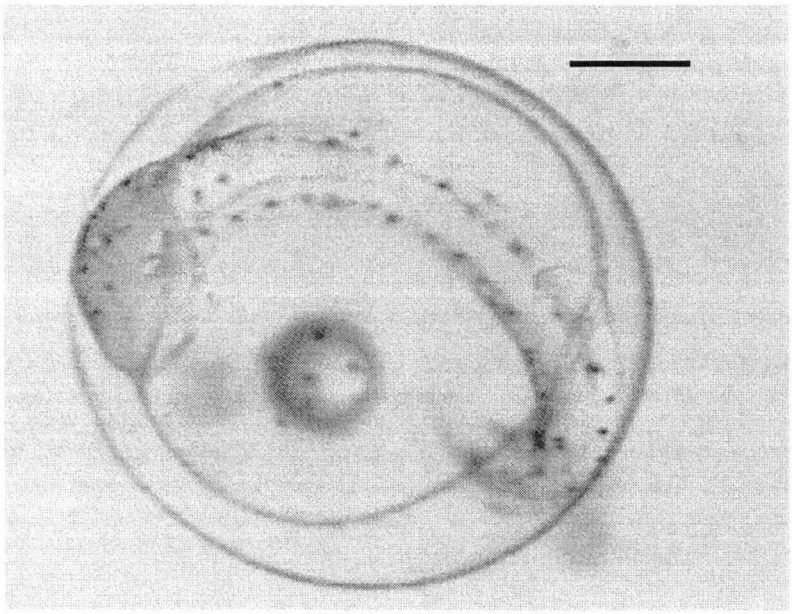

Figure 2.2. Photomicrograph of an embryo of *Notolabrus tetricus*, 42 hours after fertilisation, at a rearing temperature of 17.1-18.6 °C. Note melanophores on the inside surface of the oil droplet. Scale bar = 200 μm.

No *N. tetricus* larvae survived more than 24 hours post-hatching under any temperature regime. *Notolabrus fucicola* larvae survived to a maximum of 9 days post-hatching when reared at 11 °C (3.27 mm SL, n = 1) and 3 days post-hatching when reared at ambient temperature (2.94–3.11 mm SL, n = 5). At this stage, the yolk sac and oil droplet were mostly absorbed (Fig. 2.3b). Pectoral fin buds were present, the gut had begun to develop, with the anus open, and the eyes were developing pigment. Body pigmentation was reduced considerably from newly hatched larvae, with stellate melanophores on the dorsal surface of the developing hindgut, and paired dorso-ventrally, at the middle of the tail and adjacent to the last two myomeres. Small melanophores were scattered over the head and on the dorsal and ventral surface between the head and the anus. Small villi were evident on the margin of the dorsal and ventral fin fold.

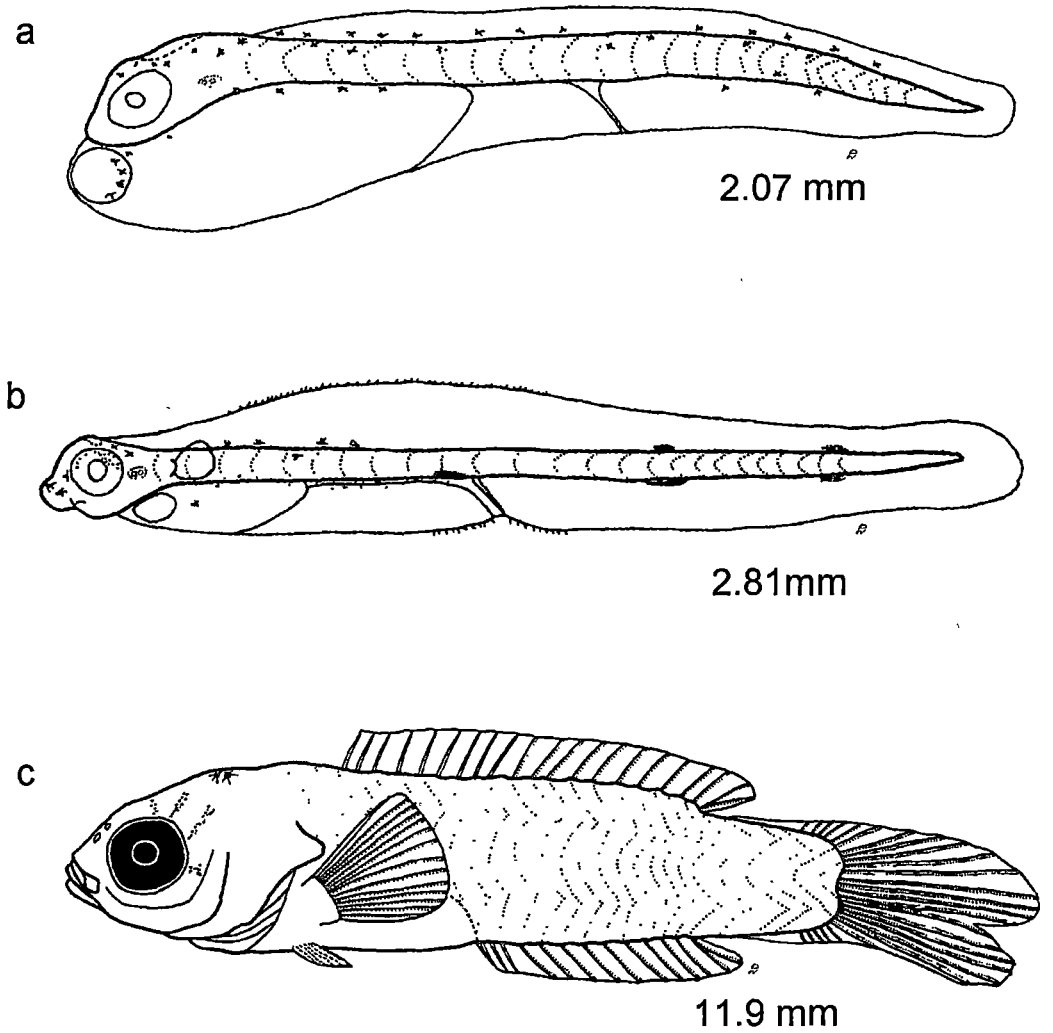

Figure 2.3. Early larvae and a recently settled juvenile of *Notolabrus* spp. **a)** 1 day old yolk sac *Notolabrus tetricus*, reared at 17.1 - 18.6 °C **b)** 3 day old yolk sac *N. fucicola*, reared at 17.1-18.6 °C **c)** *N. fucicola* post-settlement juvenile; note lack of body pigmentation, and developing pigment radiating from the orbit. The pectoral fin covers pigmentation visible through body wall, over the dorsal midgut.

Wild caught larvae

A total of 86 larvae identifiable as labrids were sorted from archived ichthyoplankton samples. Two distinct pigmentation morphotypes were evident, with type 1 being represented by 72 individuals of all developmental stages from pre-flexion through to

large post-flexion individuals. The 14 larvae of the other morphotype (type 2) were present as pre-flexion through to post-flexion larvae. The smallest individuals of type 1 (Fig. 2.4a) closely resembled the oldest reared *Notolabrus* spp. yolk-sac larvae (Fig. 2.3b). A developmental series tracking these pigment patterns resulted in the largest post-flexion individuals (Fig. 2.4e) closely resembling newly settled *Notolabrus* spp. juveniles (Fig. 2.3c) as confirmed through RFLP analysis.

Pigmentation

The smallest type 1 larvae (2.71 – 4.02 mm SL) showed a pair of stellate melanophores on the ventral mid-line on the foregut (Fig. 2.4a). This stage was connected to the following pre-flexion stage in series through individuals (3.71 – 4.26 mm SL) where a stellate melanophore had migrated up the left side of the foregut (Fig. 2.4b). This melanophore appeared to move onto the internal dorsal surface of the foregut (Fig.2.4c), where it remained visible through the abdominal wall in post-flexion individuals.

External pigmentation decreased further at notochord flexion, with both of the paired dorso-ventral melanophores disappearing from the dorsal and ventral fin fold. One or two melanophores persist in some individuals on the membrane between the fin rays in the middle of the caudal fin (Fig. 2.4d). A single stellate melanophore persists on the ventral midline in front of the anus post-flexion, along with a group of large stellate melanophore on the dorsal hindgut (Fig. 2.4e).

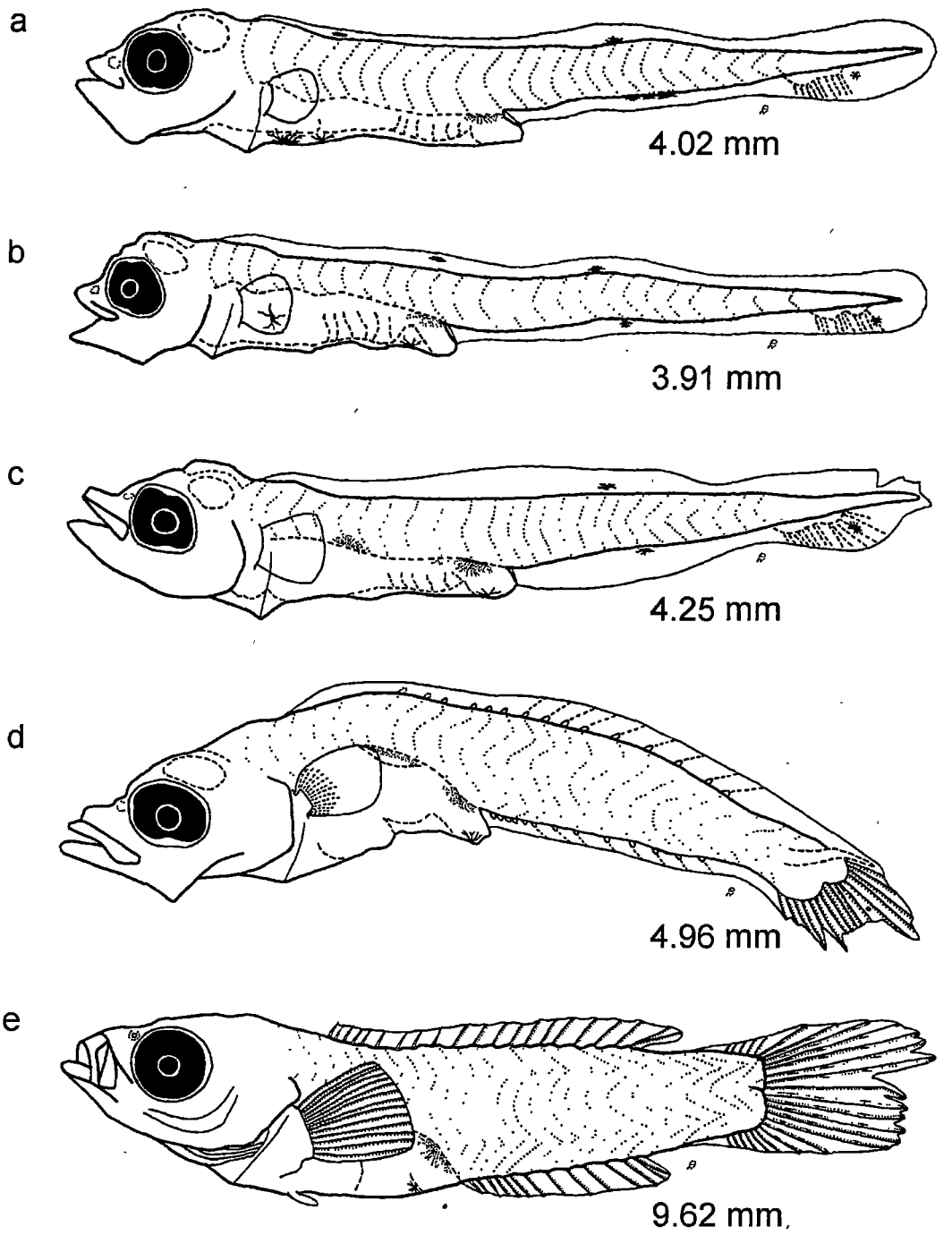

Figure 2.4. Larvae of labrid type 1. **a)** Preflexion; note melanophores on ventral midline of midgut **b)** Preflexion; note melanophore migrating up side of midgut, and melanophore near anus **c)** Preflexion; note internal pigment on dorsal midgut **d)** Flexion; note developing pectoral, dorsal and anal fin rays **e)** Post-flexion, just prior to settlement; note near absence of body pigmentation and pelvic fin bud.

Type 2 larvae are distinguished from type 1 by having larger dorso-ventrally paired melanophores present on the tail that persist in the largest post-flexion individuals (Fig. 2.5). Type 2 larvae showed 2 - 7 large punctate melanophores around the tip of the notochord, which persisted throughout development as a group on the caudal peduncle in some post-flexion individuals. Abdominal pigment consisted of 2-5 small melanophores on the external hindgut and large stellate melanophores on the internal dorsal surface of the hindgut. Type 2 larvae did not show any internal pigmentation on the dorsal surface of the foregut, in contrast to type 1 (e.g. Figs2.4c,d).

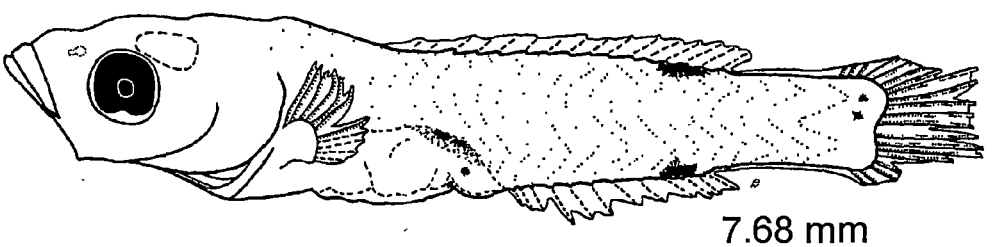

Figure 2.5. Post-flexion larvae of labrid type 2. Note large stellate melanophores at the bases of the posterior anal and dorsal incipient fin rays. Internal pigmentation is restricted to the dorsal surface of the hindgut.

Morphology

Developmental stages of both types were overlapping in size and proportions (Tables 2.2 and 2.3). Larval body shape was initially elongate, becoming deeper post-flexion. The head was small to moderate pre-flexion, increasing to moderate to large post-flexion. The gut was initially straight, with the hindgut partially striated, and a constriction just anterior to the anus. The gut was moderate to long, and coiled at flexion, becoming more compact. A moderate gas bladder was evident over the midgut in larvae undergoing flexion (e.g. Fig. 2.4d), but was covered by myomeres in larger post-flexion specimens.

Table 2.2. Summary of fin development and major developmental stages in *Notolabrus* spp., and type 1 and type 2 labrid larvae. All sizes are standard length, measured from the anterior tip of the snout to the posterior tip of the notochord (preflexion) or hypural crease (post-flexion).

Developmental stage	Size range (mm)		
	Notolabrus spp.	Type 1	Type 2
Hatching, yolk sac larva	1.70 – 3.25	-	-
Feeding, pre-flexion larva	-	2.71–5.23	4.65–4.85
Notochord flexion	-	3.75–5.78	4.01–5.26
Settlement	≥ 10.4	-	-
Dorsal and anal fin	-	3.75–9.62	4.01–7.86 +
Pectoral fin	-	2.75–9.62 +	3.94–7.86 +
Pelvic fin	10.4 +	5.29–9.62 +	7.86 +
Caudal fin	-	3.75–9.62	3.94–7.86
Scales	10.4 +	-	-

Fin development followed the same sequence in both types (Table 2.2). Pectoral fin buds were evident in the smallest type 1 and 2 larvae, with rays beginning to ossify post-flexion, and spines small or absent in even the largest post-flexion individuals, making diagnostic pectoral fin ray counts impossible. In type 1 flexion began at 3.75 mm and was completed at 5.78 mm, with principal caudal fin rays fully ossified by 9.62 mm. Minimum size at flexion in type 2 larvae was 4.01 mm, with a single 7.68 mm individual having completed flexion, with principal caudal fin rays that were ossified. In both types, fin ray and spine bases in the dorsal and anal fins were conspicuous and developed first in the posterior portion of these fins. Dorsal and anal fin rays developed in the same order, with the anterior-most dorsal spines developing last. Pelvic fin buds were evident in large post-flexion larvae.

Table 2.3. Range of body dimensions of labrid types 1 and 2, represented as percentages relative to standard length. Definitions of body dimensions follow Neira *et al.* (1998).

		% of Standard length	
Developmental stage	Body dimension	Type 1	Type 2
Pre-flexion	Body depth	12-20	13-21
	Head length	12-27	18-27
	Pre-anal length	44-60	40-57
Post-flexion	Body length	18-25	15-23
	Head length	26-39	25-37
	Pre-anal length	41-60	42-61

Post-settlement *Notolabrus* spp.

Notolabrus spp. settled at a minimum size of 10.4 mm SL (Table 2.2), and were mostly unpigmented, some with 1-3 melanophores on the nape, and/or a single melanophore in front of the anus. Post-settlement, individuals began to form lines of small pigment spots radiating back from the orbit (Fig. 3c). Body colour *in vivo* was initially translucent yellow, developing to lime green, yellow, blue gray or red brown in both species, with a silver-white stripe extending from the orbit across the opercle, and on to the pectoral fin base and the abdomen. The pelvic fin elements were not fully ossified at settlement. Scales developed at approximately 15.0 mm SL, rapidly covering the body, and formed last on the head and cheeks.

DNA extraction and PCR amplification

Extraction and amplification of DNA from adult tissue, derived from 12 adults of each species, was successful in all but one *Notolabrus fucicola*. Of the 76 post-settlement juvenile *Notolabrus* spp. attempted, 62 had DNA successfully extracted and amplified. No larval samples produced DNA that could be amplified. Therefore the genetic identity of the two larval types described could not be established.

RFLP analysis

For the three restriction enzymes tested, 11 different haplotypes were observed from the PCR reactions, including the approximately 1000 bp uncut fragment (Fig. 2.6). Of these, each enzyme produced four haplotypes across the five species tested, and 11 species-specific, composite haplotypes. *Hinf* I appeared to have no restriction site in the PCR product amplified from the single *Pictilabrus laticlavius* trialled. Restriction digests of 62 post-settlement *Notolabrus* spp., using *Bsl* I, identified 51 *N. fucicola* (haplotype A, Fig. 2.6) and 11 *N. tetricus* (haplotype B, Fig. 2.6).

Post-settlement pigment character analysis

The modified simple matching metric of similarity, plotted by MDS, produced overlapping clusters between species and size groups, with some individuals from both size groups and species showing identical suites of characters (Fig. 2.7).

The frequency of individual pigment characters showed few patterns that were species- or size group-specific (Table 2.4). Presence of light-coloured pigmentation at the base of the 8th dorsal fin spine (character H), was the only character that was exclusive to individuals of one species, occurring in 4 *N. tetricus*. However character H was not present in all *N. tetricus* examined, and hence was not sufficient to separate the two species. A group of melanophores at the base of 4th dorsal spine (character F) was never present in *N. tetricus* ≥15 mm SL (n = 3), but occurred in 75 % of *N. fucicola* in the same size group (n = 25). The presence of a group of melanophores at the base of the 7th dorsal spine (character G), was more commonly seen in *N. tetricus*, being present in 38 % of individuals <15.0 mm (n = 8), as opposed being present in 4 % of *N. fucicola* of the same size group (n = 26). This relationship held, as fish size increased, with 67 % of *N. tetricus* and 32 % of *N. fucicola* showing this character in the ≥15.0 mm size group.

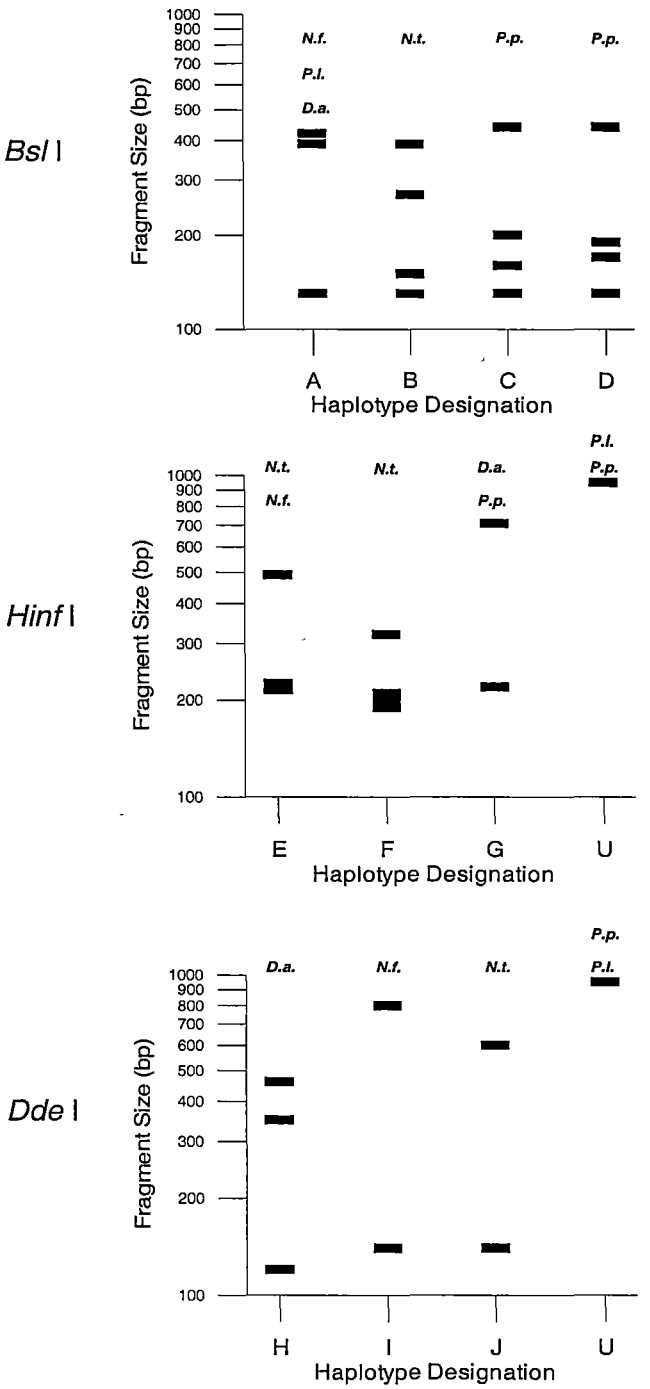

Figure 2.6. Restriction fragment length polymorphism (RFLP) haplotypes produced by three restriction enzyme digestions of an approximately 1000 base pair fragment (haplotype U) of 5 pseudolabrine wrasses. Fragment sizes are approximate, based on 100 bp ladder run alongside digests. Species abbreviations and sample sizes: *N.f.* = *Notolabrus fucicola* (*N* = 11 for *Bsl* I and *Hinf* I, *N* = 2 for *Dde* I), *N.t.* = *Notolabrus tetricus* (*N* = 12 for *Bsl* I and *Hinf* I, *N* = 2 for *Dde* I), *P.p.* = *Pseudolabrus psittaculus* (*N* = 4) , *P.l.* = *Pictilabrus laticlavius* (*N* = 1), *D.a.* = *Dotalabrus aurantiacus* (*N* = 2).

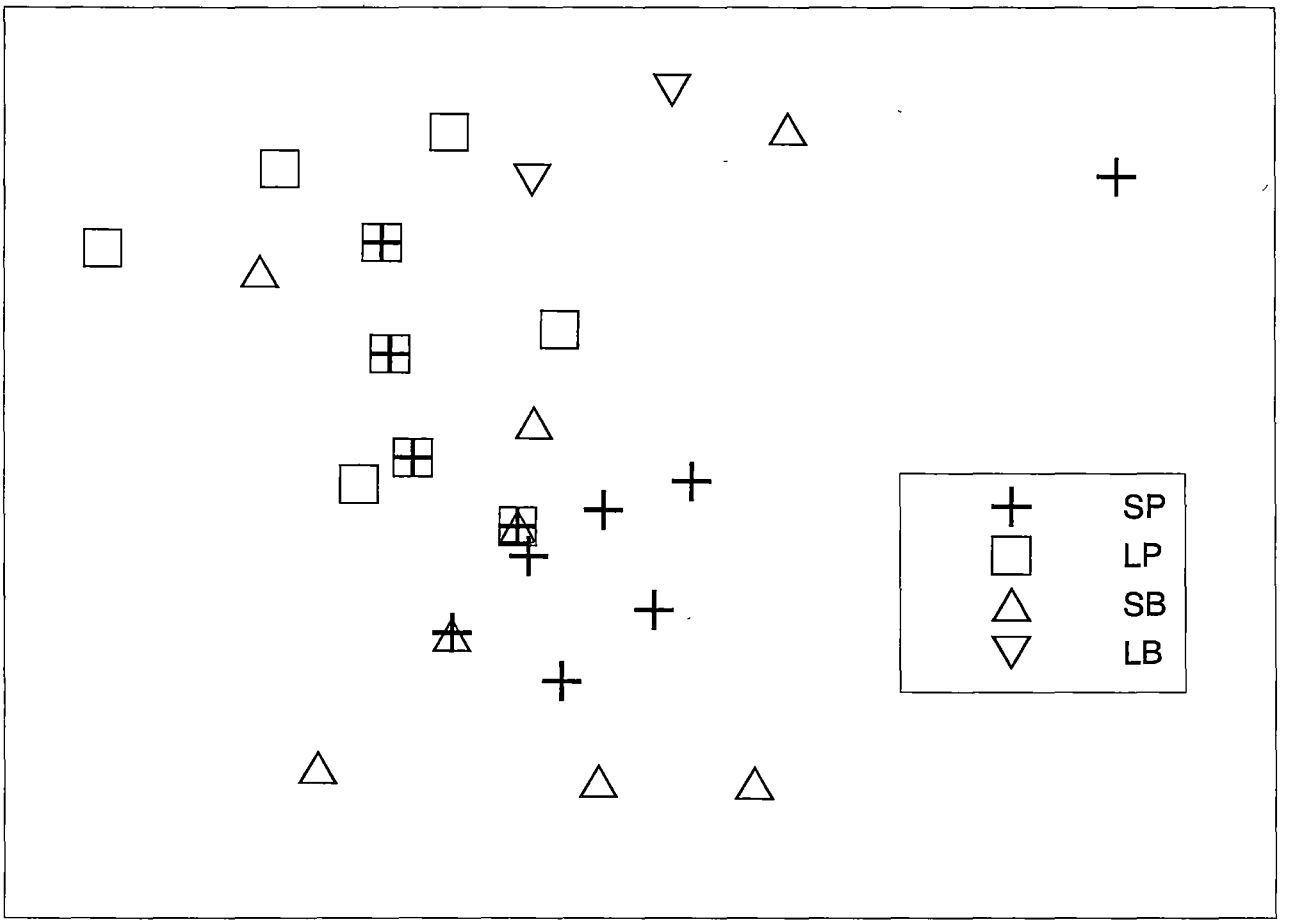

Figure 2.7. Multidimensional scaling plot based on a modified simple matching metric of similarity derived from the presence, absence or indeterminate state of 8 pigment characters in 10 *Notolabrus tetricus* and 51 *N. fucicola* post-settlement juveniles. SP = *N. fucicola* < 15.0 mm, LP = *N. fucicola* ≥ 15.0 mm. SB = *N. tetricus* < 15.0 mm, LB =*N. tetricus* ≥ 15.0 mm standard length. Stress = 0.06 after 20 restarts.

Table 2.4. Rates of occurrence of pigment characters in two size groups (S < 15.0 mm, L ≥15mm standard length) of post-settlement *Notolabrus fucicola* and *N. tetricus*, identified by *Bsl* I enzyme digest. Characters codes are described in Table 2.1.

				Character frequency (%)							
Species	Size	*N*	Presence	A	B	C	D	E	F	G	H
N. fucicola	S	26	+	12	19	15	19	15	31	4	0
			-	81	73	50	81	85	65	92	96
	L	25	+	0	48	12	4	0	75	32	0
			-	92	52	44	96	92	25	64	96
N. tetricus	S	8	+	13	13	13	29	25	25	38	25
			-	50	88	38	71	38	63	63	63
	L	3	+	0	67	0	33	0	0	67	67
			-	33	0	67	33	67	67	0	33

Discussion

Developmental series of *Notolabrus* spp.

Both species of *Notolabrus* spawned spherical, pelagic eggs, with a single oil droplet, similar to the majority of labrid genera (Leis and Rennis 1983; Richards and Leis 1984; Watson 1996). Just prior to hatching, melanophore distribution on the head and body of the embryo was similar to that seen in other wrasses (Mito 1962; Okiyama 1988; Watson 1996; Kimura *et al.* 1998). Melanophores were also present on the inside surface of the oil droplet in *Notolabrus* spp. in this study. By contrast, *Pseudolabrus* spp. from Japan have no pigmentation on the inside of the oil droplet (Mito 1962; Kimura *et al.* 1998), suggesting that this character may vary between genera within the pseudolabrine group.

Yolk sac larvae were also similar in size and appearance to other pseudolabrine wrasse larvae previously described (Mito 1962; Robertson 1973; Okiyama 1988; Kimura *et al.* 1998), with a large yolk sac that protruded forward of the head, the oil droplet at the anterior end, small villi present on the dorsal and anal fin fold, unpigmented eyes and undeveloped mouth. Pigmentation reduced considerably as the yolk sac was absorbed, until melanophores were confined to the tail and gut.

Time to hatching in *N. fucicola* and *N. tetricus* was just over two days when reared at ambient temperature (17.1–18.6 °C), whereas *Notolabrus fucicola* reared at 11°C took 5 days to hatch. This result corresponds with Robertson (1973), who reared *N. fucicola* eggs to hatching after 5 days at 10 °C. As both *N. tetricus* and *N. fucicola* spawn for an extended period over the southern spring and early summer months (Barrett 1995a), larvae will encounter a range of sea-surface temperatures through

their geographic range, and throughout the spawning season within a given region, resulting in divergent developmental rates within year classes. Hence *Notolabrus* spp. are likely to show considerable variability in parameters such as larval duration, growth and survivorship to settlement.

Two distinct series of labrid larvae, each consistent in their development of pigmentation patterns, were identified from ichthyoplankton samples sorted for this study (Fig. 2.4, 2.5). All but one labrid species on the east coast of Tasmania are pseudolabrines, and the larvae in this study closely resembled those of other pseudolabrine larvae (Mito 1962; Elder 1966; Crossland 1981; Okiyama 1988; Kimura *et al.* 1998), it seems likely that both types of larvae are from this group. Owing to their characteristic light pigmentation, in particular the complete absence of pigmentation on the head and mouth from pre-flexion until settlement, larvae in this group can now be separated from those of other morphologically similar, co-occuring fish families described from southern temperate Australia, such as the odacids (e.g. Neira *et al.* 1998).

The larvae of the types of labrid described are similar, particularly in their morphology and development (Tables 2.2,2.3), as may be expected when adults are very similar. Genetic identity of the two larval types is unknown, as no successful PCR amplification was obtained with larval samples. Therefore, there is the possibility that the larval labrid types illustrated in Figs 2.4 and 2.5 overlap one or more of the pseudolabrine genera present in Tasmanian waters. However, because the smallest and largest type 1 larvae show serial development of pigmentation patterns that are consistent with *Notolabrus* spp. (as yolk-sac larvae, and post-settlement juveniles), it seems most likely that the series illustrated in Fig. 2.4 constitutes *Notolabrus* spp. In particular, Type 1 larvae develop pigment on the dorsal midgut

(Figs 2.4c-e), the same as that seen in post-settlement *Notolabrus* spp. juveniles, and type 1 larvae post-flexion have no melanophores in the hind portion of the dorsal or anal fins (Figs 2.4d and 2.4e), and nor do post-settlement *Notolabrus* spp.

The two *Notolabrus* spp. found in Tasmania are shown in this study to have no species-specific pigmentation as early larvae or recently settled juveniles, so the possibility that type 1 and type 2, distinctive in their pigmentation patterns, are both *Notolabrus* spp. seems unlikely. Internal pigment is limited to the dorsal surface of the hindgut in type 2 larvae, unlike type 1 and *Notolabrus* spp. specimens. Paired melanophores in the tail are not seen in recently settled *Notolabrus* spp. (Fig. 3c), nor in the late post-flexion larvae and post-flexion juveniles of *N. celidotus* illustrated by Elder (1966), supporting the conclusion that type 2 larvae are not *Notolabrus* spp. A possibility for the identity of larval type 2 is *Dotalabrus aurantiacus* which can be identified, when <15 mm SL, by paired pigment patches on the posterior dorsal and anal fin rays, and at the top and bottom of the caudal peduncle extending onto the caudal fin (Welsford unpub. data). Larval type 2 also closely resembles *Pseudolabrus* spp. larvae reared in Japan (e.g. Okiyama 1988), and therefore may be the Tasmanian representative of this genus, *P. psittaculus*.

RFLP Analysis

In this study, an approximately 1000 bp fragment incorporating the D-loop region of mtDNA was successfully amplified from most developmental stages, except those specimens from archived ichthyoplankton samples. Individuals from all five species of pseudolabrine wrasses tested showed species-specific composite haplotypes when this fragment was digested with the 3 restriction enzymes applied in this study.

Sample sizes for *Pictilabrus laticlavius*, *Dotalabrus aurantiacus* and *Pseudolabrus psittaculus* were small, so larger samples sizes would increase

confidence that the same haplotypes are not shared among species. On the basis of these results however, any of the five pseudolabrine species present in Tasmania may be identified by RFLP analysis using three enzymes. This assay constitutes a foundation upon which more comprehensive analyses of pseudolabrine genetics, for identification and phylogeny, could be built.

One enzyme alone, *Bsl* I, was sufficient to reliably discriminate small post–settlement *Notolabrus* spp. juveniles at the species level (Fig. 2.6). This test was particularly useful in the context of resolving pigment character analysis of post-settlement *Notolabrus* spp., which alone did not provide a conclusive method of discriminating the two species. Thus, the two species can now be reliably identified in further studies of the early life-history of *Notolabrus* spp. that are otherwise taxonomically cryptic.

The molecular assay developed in this study had the potential to be particularly powerful in determining if the larval morphotypes described from archived samples actually reflect species or genera differences, and determine species identities conclusively. However, no individual sorted from archived ichthyoplankton was successfully assayed. It is not known why the larvae in this study could not be as readily analysed as post-settlement and adult fishes. Several studies have successfully compared adult and larval genetics without modifying extraction or amplification protocols to account for smaller tissue sample sizes from larvae (Hare *et al.* 1994; Lindstrom 1999). It may be in some part due to storage of specimens for 10+ years at ambient air temperatures resulting in tissue degradation. More recently captured specimens may prove suitable for analysis with the protocol described.

Post-settlement pigmentation analysis

Post-settlement juveniles of both *Notolabrus* spp. in this study had variable expression of the pigment characters scored. No individual or suite of characters provided a method for reliably identifying juveniles of these species. As evidenced by the MDS plot (Fig. 2.7), many individuals of both species had identical scores, and small individuals tended to be more alike in character frequencies than larger individuals when contrasted across species.

The presence of white pigment granules at the base of the 8th dorsal fin spine was found only in *N. tetricus*, and so this character is diagnostic if present. However, this character is not present in all individuals of this species. Similarly, the presence of melanophores at the base of the fourth dorsal fin spine was exclusive to *N. fucicola* in the ≥15.0 mm size group. As only 3 *N. tetricus* ≥15.0 mm were available to compare with 26 *N. fucicola,* there is insufficient power to determine if this character is broadly species-specific. Both species form scales at around 15.0 mm, simultaneous with increasing pigmentation. Therefore, juveniles much above this size can be identified by counting cheek scales, or looking for adult colouration patterns. This study suggests therefore, that a limited number of recently settled *Notolabrus* spp. juveniles <15.0 mm SL are likely to be discriminated on the basis of pigmentation patterns, and the RFLP analysis developed by this study is a more reliable method.

Many groups of fishes are morphologically and meristically conservative, while being ecologically and geographically diverse. In cases were species are not distinguishable by geographical ranges or external features, it can be a difficult task to identify species accurately, particularly in early life-history stages where species-specific adult characters such as pigmentation, scales or sexual dimorphism are undeveloped. These difficulties are compounded by larval and juvenile fishes

undergoing rapid size and ontogenetic changes, along with habitat shifts. This situation is certainly the case for pseudolabrine wrasses, which manifest the difficulties encountered in early life-history studies of labrids in general (e.g. Hare *et al.* 1994), and in other morphologically conservative groups of fishes (e.g. *Sebastes* spp.: Nedreaas and Nævdal 1991; lutjanids: Schultz *et al.* 1996; gobiids: Lindstrom 1999). In these circumstances, it is required to use molecular-based techniques to establish relationships between adult and early life stages.

As *Notolabrus* spp. are home-ranging as adults (Barrett 1995b), and have low mortality in the adult phase (Barrett 1995a), adult population dynamics are likely to be influenced by factors operating on the early life stages. Accurate assessment of the relationships between life stages requires accurate identification of all life stages to the lowest level possible. This study represents the first illustration and description of the larval development of *Notolabrus* spp. and other pseudolabrine wrasses from temperate Australian waters, and enables the identification of early life stages in this group for future studies.

3. Interpretation of otolith microstructure in the early life- history stages of *Notolabrus fucicola* and *N. tetricus*.

In press as Welsford, D.C. (2003).Interpretation of otolith microstructure in the early life-history stages of *Notolabrus fucicola* and *N. tetricus*. *Marine and Freshwater Research*

Abstract. *Notolabrus fucicola* and *N. tetricus* larvae were reared in the laboratory at a constant temperature of 11°C and both species hatched after 4 days. Mortality was high throughout the post-hatching stage, with no *N. tetricus* surviving beyond 4 days post-hatching. *Notolabrus fucicola* late yolk-sac larvae showed no clear incremental structure in their sagittal otoliths up to 9 days post-hatch. The radius of the otoliths of these late yolk-sac larvae corresponded with the radius of a non-incremental region around the primordium of sagittae taken from post-settlement individuals of both species captured in the wild. Therefore, it is likely that the first increment is formed at or near yolk-sac absorption in these species. Post-settlement juveniles of both species were exposed to oxytetracycline (OTC) and held in aquaria for up to 33 days. Post-OTC increment counts showed that increments were formed daily in the sagittae of both species.

Introduction

The interpretation of otolith microstructure to age young fish is a powerful tool, providing insights into the timing and rate of many processes acting during early life. There are three essential requirements for a reliable ageing protocol: 1) The ability to detect some sort of periodic structure in the otolith (i.e. ring/increments), 2) validation that periodic structure reflects some external time scale (e.g. rings are laid down daily), and 3) determination of the time when periodic structures begin forming in the larval otolith (Jones 1986; Campana 2001).

Many studies of larval and juvenile fish have established that some kind of daily increment structure is present in the otoliths (see Jones, 1986 for review). However, exceptions exist, such as species where growth rates are low (e.g. Secor and Dean 1989), or that live in cold water (e.g. Casas 1998), forming less than one increment per day. In some tropical and fast growing species, more than one increment may be observed ('sub-daily increments' e.g. Victor 1986; Prince *et al.* 1991).

A common method for establishing the temporal pattern of visible increments involves marking otoliths with a chemical tag such as oxytetracycline (OTC), holding animals in captivity, and then comparing the number of increments observed in the otolith with the known period after OTC exposure (Campana 1983; Fowler 1989; Secor and Dean 1989; Hoedt 1992; Lou and Moltschaniwskyj 1992; Sogard *et al.* 1992).

Determining the timing of the first increment is critical to establish a baseline from which ages are calculated (Campana 2001). This usually requires rearing larvae (Jones 1986), or having wild caught specimens of known age (Jenkins 1987; Jordan 1994). Fishes generally begin to form visible daily increments coincident with other

developmental landmarks; at or near hatching, exhaustion of yolk supplies or onset of exogenous feeding (Jones 1986). Determining the exact timing of the onset of daily increment formation can be difficult, as discerning the very first increment may be limited by sample preparation (Campana and Neilson 1985) and the resolution of available microscopy (Campana *et al.* 1987).

Much of the work on the early life-stage ecology of wrasses (Pisces: Labridae) has been conducted on tropical species, with parameters such as planktonic larval duration and age estimated from the increments observed in otoliths (e.g. Victor 1982; Victor 1986; Victor 1987; Sponaugle and Cowen 1997; Robertson *et al.* 1999; Wilson and McCormick 1999; Victor and Wellington 2000). There have been relatively few studies of this nature concerning temperate species (see Cowen 1985; 1991; Sogard *et al.* 1992 for exceptions). A few studies have directly validated the daily nature of increment formation in tropical and temperate wrasse species (Victor 1982; Sogard *et al.* 1992). Many of the studies utilising otolith microstructure in the early life-stages of wrasses are limited, however, by the lack of determination of the timing of the initiation of daily increments. It is usually assumed that fish age is calculated as the count of increments plus two (e.g. Victor 1986; Cowen 1991; Sogard et al. 1992). This assumption, largely untested, stems from Victor's (1982) observation that some tropical wrasse species form otoliths at two days after fertilisation, coincident with hatching, and daily increments begin immediately thereafter.

Embryonic development in tropical and warm temperate wrasses is rapid, species with pelagic eggs hatching in 18-34 hours when reared at temperatures >20 °C (Mito 1962; Kimura *et al.* 1998), and larvae may commence exogenous feeding within 42 hours after hatching (Colin 1982). This contrasts with development rates of the benthic spawning temperate wrasse, *Labrus merula*, taking up to 4 days to hatch at

a rearing temperature of 14.3 °C (Dulčić *et al.* 1999). Illustrations of larval wrasses often depict the otic capsule formed and containing visible sagittae and lapilli before the embryo has hatched (e.g. Kimura *et al.* 1998), contrasting with Victor's (1982) observations. Hence the timing of otolith formation and commencement of daily increments may vary considerably with environmental temperature and rate of development in wrasse embryos and larvae.

The purple wrasse (*Notolabrus fucicola* Richardson) and the blue-throated wrasse (*N. tetricus* Richardson) are two commonly observed species on shallow reefs on the east coast of Tasmania. *Notolabrus fucicola* is a home-ranging, gonochoristic species, while *N. tetricus* is a protogynous hermaphrodite, with females home-ranging and males aggressively territorial during the spawning season (Barrett 1995a; Barrett 1995b). *Notolabrus fucicola* and *N. tetricus* are of increasing economic importance with the recent establishment of a live export fishery in southeastern Australia (Lyle and Hodgson 2001).

Barrett (1999) found very low levels of natural mortality in adult *N. fucicola* and *N. tetricus*, and suggested that population structure may be influenced by factors operating in the early life-history stages (i.e. planktonic larvae, post-settlement juveniles). The early life-history ecology of this genus is unknown, apart from work on demersal *Notolabrus celidotus* (Bloch & Schneider) juveniles of in New Zealand (Jones 1983; 1984). Jones was unable to establish an accurate daily ageing method for juvenile wrasse by examining scales. Therefore, a first step in further elucidating the early life-history ecology of these species is a reliable protocol for ageing. This study describes the validation of daily increment formation in the otoliths of post-settlement *N. fucicola* and *N. tetricus*, and establishes the onset of visible increments at yolk sac absorption.

Materials and Methods

Larval rearing

Gamete collection and fertilisation

Adult wrasses were caught during daylight using hook and line over shallow reef on the east coast of Tasmania. Fish were identified to species, stripped for gametes if running ripe and then released immediately. Adult *Notolabrus fucicola* were sampled on 30/10/1998 at the northern end of Maria Island (42.57°S, 148.07°E), and two individuals of each sex of were stripped. *Notolabrus tetricus* were caught on 10/11/1998 at Yellow Bluff (43.14°S, 147.40°E), and as *N. tetricus* males were less common, one male and 2 females were stripped.

Sperm was expressed into a 25 mL plastic jar and diluted with seawater. Eggs were collected in 1 L glass jars, diluted with seawater then flushed, by pouring them onto a 250 μm sieve and gently rinsing with seawater, to remove mucus. Around 100 mL of 'dry' eggs were then resuspended in the glass jars. The diluted sperm was added to the eggs, and the jars transported in insulated containers to the laboratory.

Holding conditions

The contents of each jar were transferred to 9 L clear plastic containers and the volume was made up with filtered seawater at ambient air temperature. Temperature was maintained at a constant level of 11 °C. Artificial illumination was provided by ceiling lights on a 12:12 hr light: dark cycle in approximate synchrony with the natural diel cycle. Every 24 hours, the bottom 90% of the tank volume was siphoned off, including non-viable eggs and dead larvae. The volume of the container was then refilled with filtered seawater at the trial temperature.

Post-settlement fish

Field sampling

Post-settlement *N. fucicola* and *N. tetricus* were obtained from three sites on the east coast of Tasmania, Bicheno (41.82°S, 148.30°E), Recherche Bay (43.59°S, 146.92°S) and Okehampton Bay (42.53°S, 147.98°E) (Table 3.1). At each site, fish were collected after they had settled on artificial habitats (Chapter 4). After capture, they were placed in a 100 L plastic barrel, containing seawater at ambient air temperature (15 – 20 °C), for transport back to the laboratory within 48 hours.

Table 3.1. Summary of dates and numbers of wrasse exposed to OTC to validate periodicity of formation of otolith increments.

Species	*N*	Site	Date captured	Days held
Notolabrus fucicola	15	Bicheno	14/12/2000	8
N. fucicola	1	Bicheno	18/01/2001	33
N. fucicola	11	Recherche Bay	21/01/2001	30
N. tetricus	1	Okehampton Bay	18/01/2001	33
N. tetricus	1	Okehampton Bay	31/01/2001	19
N. tetricus	1	Okehampton Bay	31/01/2001	26
N. tetricus	4	Okehampton Bay	27/02/2001	28

Oxytetracycline staining

To stain their otoliths, a sub-sample of post-settlement fish were placed in a solution of 500 $mg.L^{-1}$ of oxytetracycline (OTC) in seawater (2.5 $mL.L^{-1}$ of Ilium Oxytet-200 L.A., containing 200 $mg.mL^{-1}$ OTC as the base) for 24 hours. This concentration has been shown to produce reliable marks in juveniles of the temperate labrid *Tautoga onitis* (Sogard *et al.* 1992).

Holding conditions

Fish were removed from the OTC solution to 20 L plastic buckets with seawater flow-through at 2 $L.min^{-1}$. Seawater was at ambient temperature (15-17 °C). All the fish caught on a particular sampling date were held in a single bucket (Table 3.1). Each bucket contained shelter (a concrete brick and a piece of black plastic mesh). Buckets were housed outside under cover, exposed to ambient natural light cycles. Fish were fed *Artemia* spp. *ad libitum* (~$100.fish^{-1}$) daily.

Identification, measurement and curation

Post-settlement fish and reared larvae were euthanased in 2 $g.L^{-1}$ benzocaine in seawater, before being preserved in absolute ethanol. Samples of reared larvae were collected daily after hatching. Post-settlement fish captured for inspection of otolith microstructure were euthanased as soon as they reached the laboratory. Post-settlement fish held for determination of periodicity of increment formation were euthanased at different times after immersion in OTC (Table 3.1). Post-settlement fish were identified to species, and the total length (TL), from the anterior tip of the snout to the middle of posterior margin of the caudal fin, was measured to the nearest 0.1 mm, using vernier calipers.

Otolith analysis

Preparation and increment reading

Sagittae were extracted under a binocular dissector, using cross-polarised transmitted light, and were embedded, sulcal side down, in thermoplastic resin on a glass microscope slide. Larval otoliths were mounted whole whereas otoliths from post-settlement fish were ground using 1200 grade wet and dry sandpaper until increments were clearly visible. Increment structure in post settlement fish was most clear when otoliths were ground, in the sagittal plane, flipped over onto the ground facet, and

ground again. Ground facets were polished using alumina powder wetted into a paste and spread on a synthetic felt surface. The uppermost polished facet was finally covered with a thin layer of thermoplastic resin.

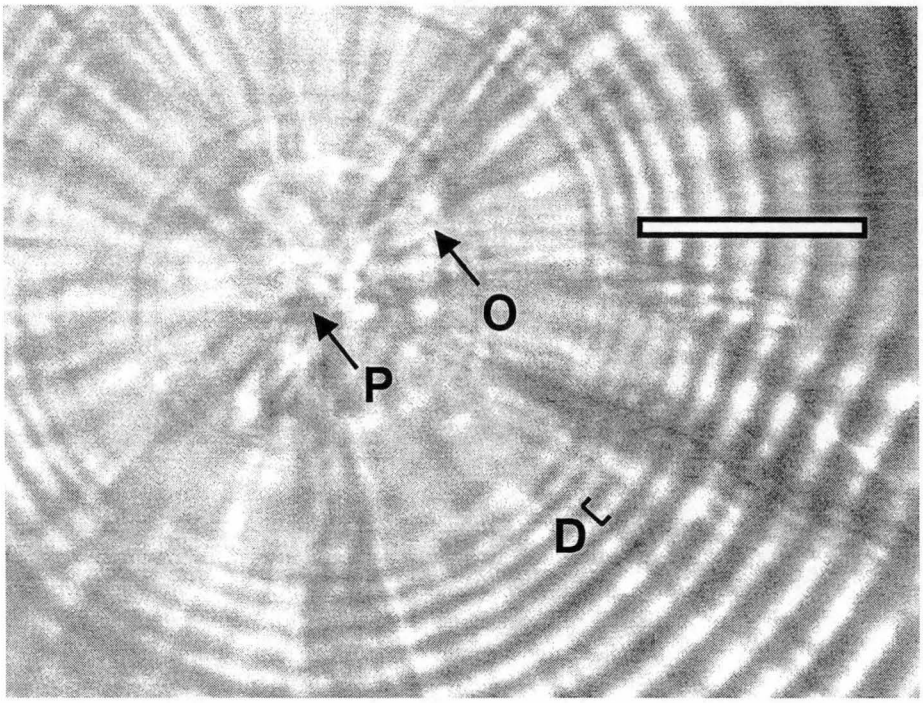

Figure 3.1. Photomicrograph of a ground and polished sagitta from a post-settlement *Notolabrus fucicola.* P= Primordium, O= Position of onset of daily increments, D= typical daily growth increment. Scale bar =10 μm.

Increments were clearly visible under transmitted illumination at ×400 magnification as a typical incremental and discontinuous zone pair (*sensu* Campana and Neilson 1985) (Fig. 3.1). A single increment was counted as the inner discontinuous zone and outer incremental zone. The total number of increments from the first visible increment near the primordium to the otolith edge was counted in all sagittae exposed to OTC. The repeatability of counts was verified within and between individual readers (Chapter 4).

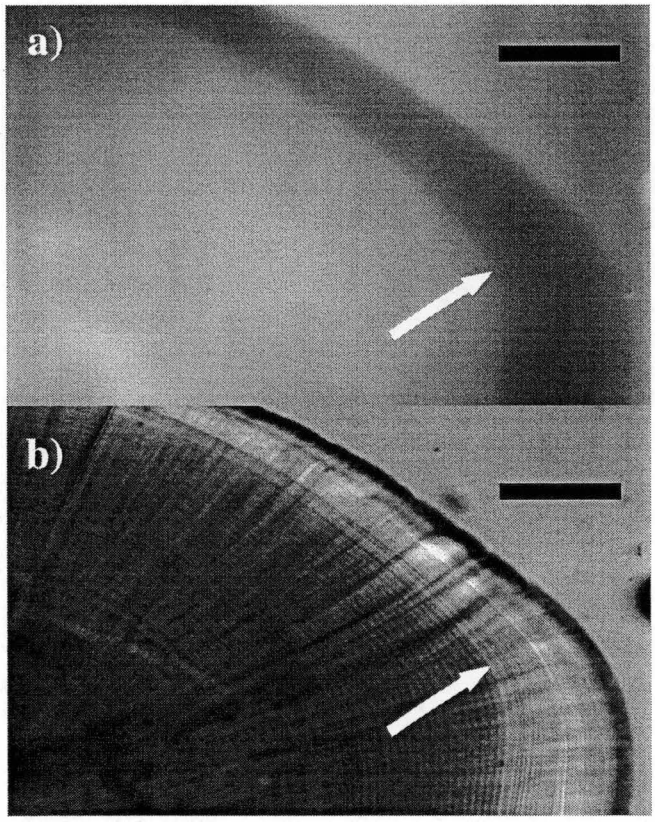

Figure 3.2. a) Photomicrograph of the margin of the sagitta of a post-settlement *Notolabrus fucicola* exposed to OTC, as seen under UV illumination. Arrow indicates outer margin of material fluorescing due to OTC. Scale bar = 50 μm. **b)** The same otolith seen under transmitted normal light. Arrow indicates check mark coincident with the outer margin of OTC stained material. Scale bar = 50 μm.

The margin of fluorescence due to OTC (Fig. 3.2 a) was visualised using a Nikon Optiphot microscope (×400), with fluorescent light supplied by Nikon episcopic-fluorescence attachment EF-D with a 100 W mercury lamp. Fluorescent marks in all otoliths were associated with a check visible under normal illumination (Fig. 3.2 b). Consequently, increments were counted under normal illumination from the check to the edge of the otolith. The check was included in the count as the inner incremental zone of the first post-fluorescence increment. The outer edge of the otolith was not included as part of an increment as it consistently appeared opaque.

Measurement

Images of sagittal otoliths were digitised and measured using image analysis software (Scion Image beta 4.0.2 http:\\www.scioncorp.com). All post-settlement otoliths were first digitised at ×40, and the radius from the primordium along the axis of maximum growth recorded to the nearest μm. For 15 post-settlement individuals of each species that had not been exposed to OTC, the primordial region was then digitised at ×400 and the maximum radius out to the edge of the first visible increment (Fig. 3.1) measured to the nearest 0.1μm. Images from sagittae of larval fish were digitised at ×400 and the maximum radius measured to the nearest 0.1 μm.

Results

Larval rearing

Eggs of both species were of similar size (0.98 - 1.09 mm diameter, N =10), and yolksac larvae were similar length at hatching (6 *N. fucicola,* 1.7 - 2.1 mm TL, 4 *N. tetricus*, 1.93-2.11 mm TL). Eggs of both species hatched after 4 days when reared at 11°C. Mortality was high throughout the post-hatching stage, with no *N. tetricus* surviving beyond hatching, and very few *N. fucicola* surviving to 9 days post-hatching, at which time the trial was terminated. Inspection under a stereomicroscope (×25) showed that the larvae at 9 days post-hatching had absorbed the majority of their yolk, jaws were partially developed and the eyes had started to develop pigment.

Onset of increments

Five *N. fucicola* yolk-sac stage larvae (5 - 9 days post-hatching) had their sagittae extracted and measured. The maximum radius of the otoliths showed an increase with the known age of the larvae (Fig. 3.3 a). The maximum radius of the primordial non-

incremental zone did not differ significantly between post-settlement *N. fucicola* and *N. tetricus* (T_{28} = -0.49, P = 0.32)(Fig. 3.3 b). The maximum radius of otoliths from the oldest *N. fucicola* larvae was very similar to the mean radius of the primordial non-incremental zone in both species.

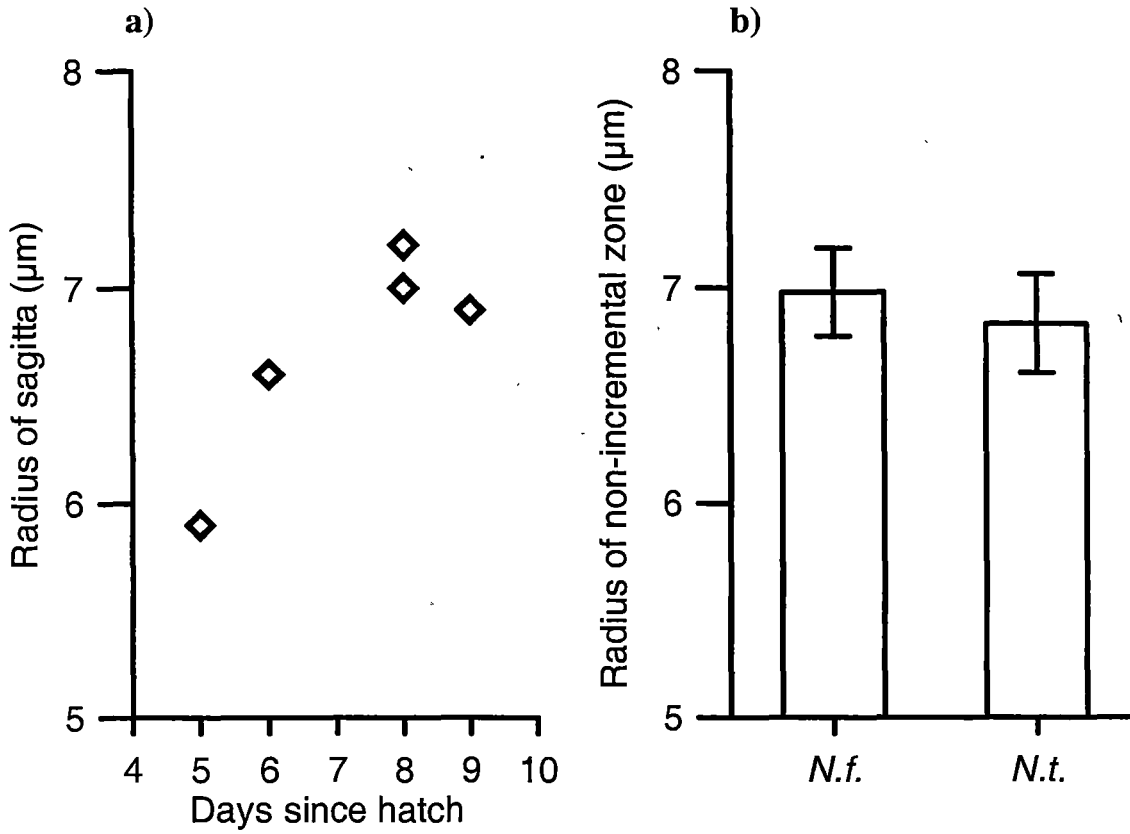

Figure 3.3. a) Radius of whole larval otoliths from reared *Notolabrus fucicola* larvae of known age. **b)** Mean size of primordial non-incremental zone in otoliths of post-settlement *Notolabrus tetricus* and *N. fucicola*. For each species N = 15. Error bars are +/- standard error of the mean.

Oxytetracycline marking

Exposure to oxytetracycline (OTC) over 24 hours did not result in any mortality in either species. After they were removed from the OTC solution, all fish survived to the planned endpoint of the experiment. All otoliths showed clear distinction between pre- and post-exposure regions in the sagittae, both under fluorescent illumination (Fig. 3.2a), and transmitted visible light (Fig. 3.2b).

Post-OTC increments

Increments beyond the OTC stained region of the sagittae in post-settlement stages of both species were similar in appearance to those laid down prior to exposure to OTC, although in some cases they appeared to be narrower (Fig. 3.2b).

In *N. tetricus*, total length (TL) of field caught post-settlement fish ranged from 22.4 to 51.9 mm and the total numbers of increments in sagittae ranged from 91 to 145. For all 7 *N. tetricus*, the number of post-OTC increments was equal to the number of days post-exposure to OTC.

Total lengths of *N. fucicola* ranged from 15.6 to 40.7 mm and the total number of increments in the sagittae ranged from 79 to 160. Of 27 *N. fucicola* exposed to OTC, 20 fish had post-OTC increment counts equal to the expected number of daily increments. Three fish showed 1 post-OTC increment less than expected on the basis daily formation, and 4 fish demonstrated 1 increment greater than expected (Fig. 3.4). A *t*-test of paired samples confirmed that the number of increments was not significantly different from daily ($T_{52} = 0.372$, $P = 0.713$). Regression analysis of the residuals from the 1:1 daily increment model revealed no significant relationship between residuals and the TL of the fish, total increment count, or maximum radius of the sagittal otolith (Fig. 3.5).

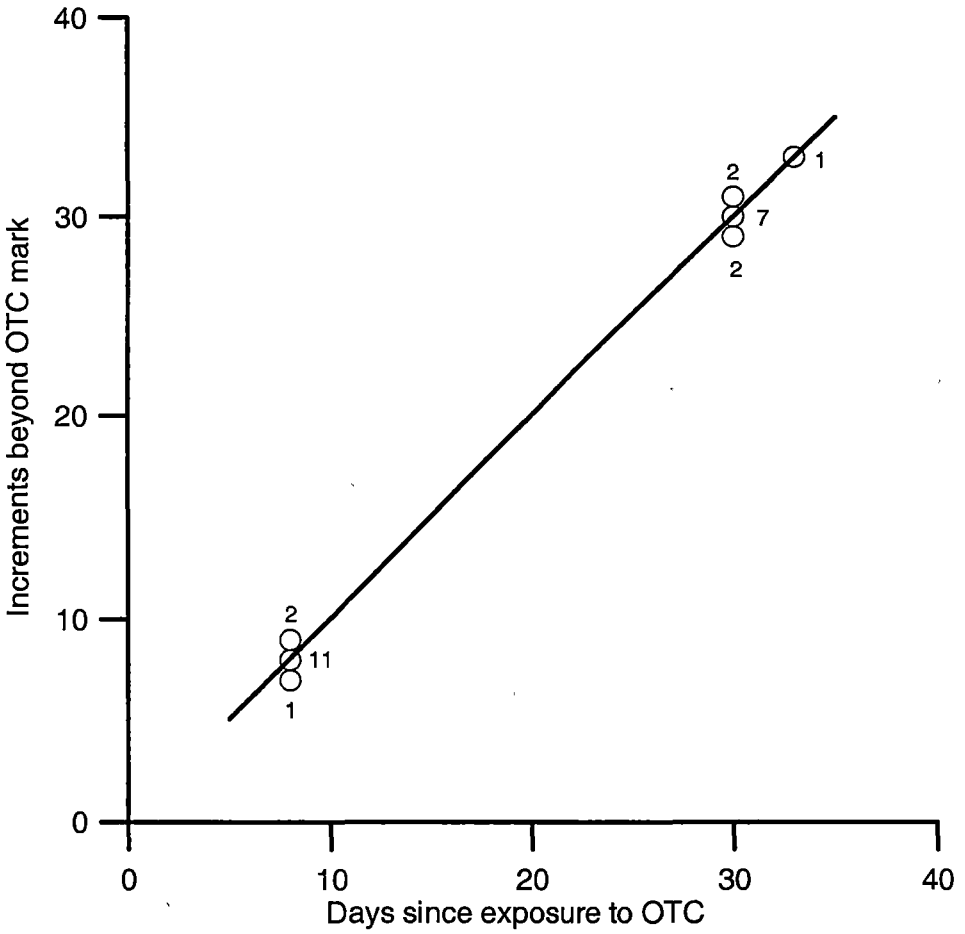

Figure 3.4. Post-OTC increments in the sagittae of post-settlement *Notolabrus fucicola* against the number of days individuals were maintained after exposure. Numbers adjacent to circle indicate number of data points at that position. Solid line shows 1:1 relationship.

Discussion

Onset of increments

The larvae of *Notolabrus fucicola* that were reared to the end of the yolk-sac phase showed no incremental structure in their sagittae. The otoliths of wild-caught post-settlement individuals of both species were also characterised by a non-incremental zone around the primordium, after which regular increments were evident (Fig. 3.1). Significantly, the mean radii of this non-incremental zone in post-settlement *N. fucicola* and *N. tetricus*, captured from the wild, were very similar to radii of sagittae

from late yolk-sac *N. fucicola* (Fig. 3.3). Although it has been found that otolith microstructures in laboratory-reared fish may not closely resemble those from specimens captured from the wild, the periodicity of increment formation is usually not compromised (Campana and Neilson 1982; Campana 1983; Campana 2001). The *Notolabrus* spp. larvae in this study were reared under diurnal lighting conditions and temperatures equivalent to those they would encounter in the coastal waters of south-eastern Tasmania during the austral spring, when these species have a peak of spawning activity (Barrett 1995a). Therefore, a region of non-incremental material, laid down during the yolk sac phase of larval life, seems to be a normal part of the development of the sagittae of *N. fucicola*, and not an artefact of laboratory rearing.

Development of the eyes, jaws, and the near exhaustion of the yolk sac in the oldest *N. fucicola* larvae suggests that exogenous feeding would have commenced within the next few days. Hence, it seems reasonable to conclude that the clear incremental structures seen in the otoliths of post-settlement *N. fucicola* and *N. tetricus* begin at or near yolk sac absorption and the beginning of exogenous feeding. Although no larvae of *N. tetricus* reached the late yolk-sac stage, the time to hatching was identical in the two species when reared at the same temperature. It is likely, therefore that the onset of visible increments in the sagittae of *N. tetricus* is also at first-feeding. This concurs with the onset of formation of daily increments at first feeding found in other temperate species with pelagic larvae from various families (Jenkins 1987; Thresher *et al.* 1989a; Jordan 1994; Sepúlveda 1994).

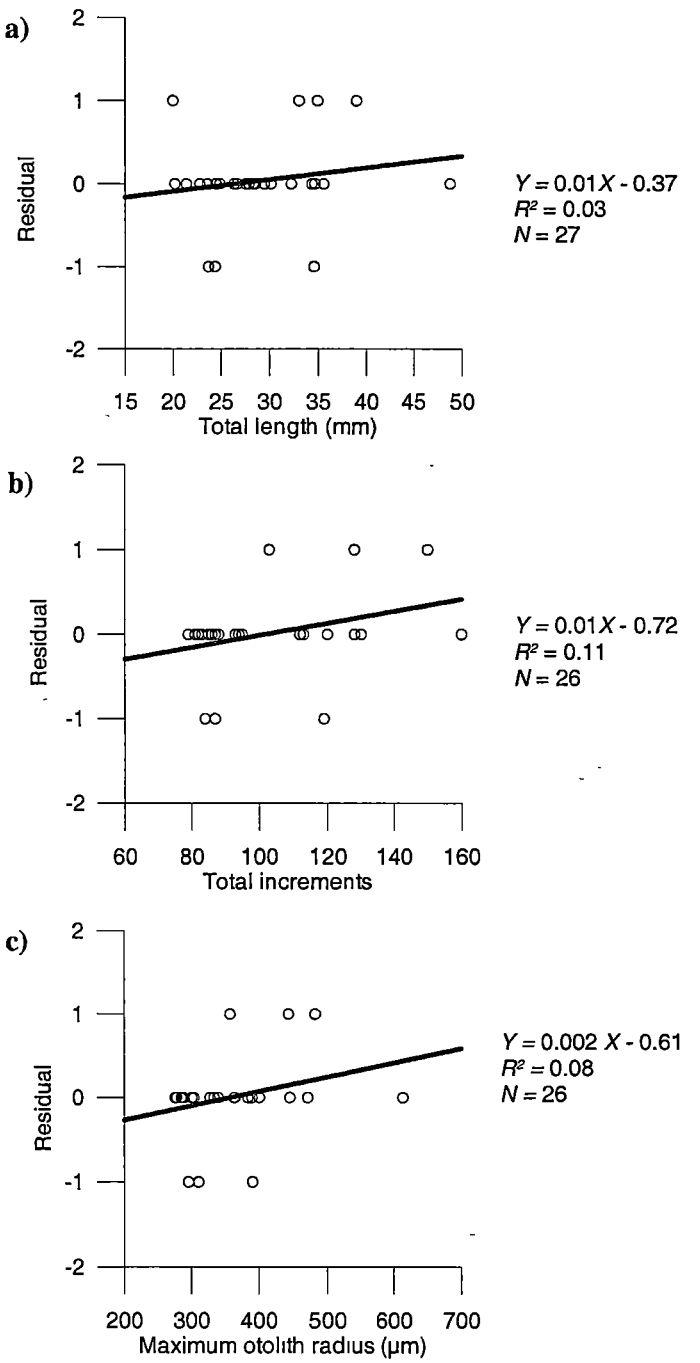

Figure 3.5. Plots of daily increment residuals for *Notolabrus fucicola* exposed to OTC against **a)** total length of fish, **b)** otolith total increment count and **c)** radius along the otolith axis of maximum growth.

At a rearing temperature of 11°C, *N. fucicola* larvae were up to 9 days post-hatching and 14 days since being fertilised when they were near first-feeding. This suggests, that to back-calculate spawning dates of *N. fucicola* and *N. tetricus*, at least 14 days must be added to the number of daily increments counted in sagittae if eggs

develop in water at 11°C. This contrasts the pattern inferred for other labrids, where onset of increments in all species was assumed to occur two days after fertilisation (Victor 1986b). That is not to say that an interval of two days from fertilisation to the formation of the first daily increment is not a reasonable approximation for wrasse larvae developing in warm temperate and tropical waters. At rearing temperatures of 20-27 °C, embryonic development in wrasse larvae from several genera is shown to be rapid, with larvae developing otoliths, hatching and progressing to exogenous feeding in as little as 3-5 days after fertilisation (Mito 1962; Colin 1982; Kimura *et al.* 1998).

Validation of daily increments

In post-settlement juveniles of *N. fucicola* and *N. tetricus*, increments are laid down daily. This is not surprising, as other temperate and tropical labrids have had daily increments validated or inferred (Victor 1986b; Cowen 1991; Sogard *et al.* 1992). Indeed, non-daily increment formation in young fishes is the exception rather than the rule (e.g. Casas 1998). However, this is the first instance of direct validation of daily increments in the sagittal otoliths of pseudolabrine species (*sensu* Russell 1988).

In both species examined, a mark visible under normal illumination coincided with the edge of OTC stained material in the otolith. Similar marks described in other studies have been attributed to the stress of the holding conditions during exposure to OTC (Fowler 1989; Hoedt 1992; Vigliola 1997). It is possible the mark is related to the relatively high dosage of OTC used in this study (500 $mg.L^{-1}$), more than stress, since exposures to OTC at a concentration of 250 $mg.L^{-1}$ for as little as 4 hours produced a similar mark in the anchovy *Thryssa aestuaria* (Hoedt 1992), and OTC is reported to affect growth of calcified structures such as otoliths (Kobayashi *et al.* 1964). Increments formed after exposure to OTC showed little fluorescence,

suggesting that OTC may not remain in the otic endolymph, or influence increment formation, beyond the period of direct exposure.

The daily nature of increment formation in these *Notolabrus* species has been directly shown for a broad range of ages (79 – 160 increments in total). Any divergence from a strict daily regime was unrelated to the size of the fish or its otolith (Fig. 3.4). Apart from a check formed at settlement, and increments becoming difficult to discern after 200+ due to otolith thickness and increment narrowing (Welsford unpublished data), there are no obvious 'hesitations' (*sensu* Thresher 1990) in the structure of these otoliths. Therefore it seems valid to infer that once daily increments begin to be laid down in the larval otolith, they form on a daily basis thereafter.

Utility of otolith microstructure in *Notolabrus* spp.

The rationale behind this study was to provide an accurate framework for the ageing of the early life-stages of *N. fucicola* and *N. tetricus*. With the validation of the daily nature of the clear incremental structure in the sagittae of these fishes, and anchoring of the onset of these increment formation to the time at or near first-feeding, (at around 14 days post-fertilisation when reared at 11°C), accurate ageing of these fishes is possible for the first 200 days of life. This will facilitate insight into growth rates, survivorship and recruitment processes in future studies of the early life-stages of these ecologically dominant and increasingly economically important temperate reef fishes.

4. Settlement dynamics of temperate wrasses on the east coast of Tasmania

Abstract. Recently settled juveniles of the temperate reef-associated labrid, *Notolabrus fucicola* were studied at two sites on the east coast of Tasmania. Fish were captured after settling on artificial habitats placed in shallow water adjacent to kelp reef habitat. Habitats were sampled approximately monthly during the settlement season (Spring- Summer) over three years, 1998-1999 through 2000-2001. Sagittal otoliths were analysed to determine age (in days) and back calculated spawning dates, which enabled the comparison of growth rates within and between year classes at each site. Daily increment counts were shown to be free of within and between reader bias, and were independent of otolith type. A check mark in the sagittae was observed in approximately 50% of individuals, and was shown to be associated with the timing of settlement, enabling relationships between spawning, settlement and plankton larval duration to be analysed. Recently settled *Notolabrus fucicola* were spawned over a range of dates with no strong relationship between spawning or settlement date and moonphase. Planktonic larval duration and size at settlement was highly variable, ranging from 40 to 87 days and 10.4 to 15.2 mm standard length respectively. Over the three settlement seasons studied, settlement peaked earlier in each successive year. This pattern corresponded with increased water temperatures off the east coast of Tasmania over the same period, particularly in 2000-2001. Significant linear relationships were found at both sites in all years between spawning and settlement date, with individuals spawned later in each year spending less time in the plankton. Post-settlement growth is also shown to increase in cohorts spawned later in each

season. However, those individuals spawned earlier each year that survived to settle retain a size advantage over all subsequent settlers. A high level of inter- and intra-annual variability and unpredictability in the nearshore planktonic environment makes maximising reproductive output difficult at small time and spatial scales. A long spawning season and flexibility in time spent in the plankton in *Notolabrus* ensures that some propagules are likely to survive to settle each year.

Introduction

Wrasses are one the most speciose and widespread of fish families, and are conspicuous members of the reef fish faunas in temperate and tropical waters. A total of eight species of wrasses are reported from the rocky coastal reefs of Tasmania (Gomon *et al.* 1994; Edgar 1997). The two largest and most conspicuous species are the purple wrasse, *Notolabrus fucicola* (Richardson, 1840), and the blue-throated wrasse, *N. tetricus* (Richardson, 1840). *Notolabrus fucicola* also occurs all around New Zealand, and the coast of south-eastern Australia from southern New South Wales west to Kangaroo Island in South Australia, while *N. tetricus* is found from Sydney to the Great Australian Bight (Russell 1988; Gomon *et al.* 1994; Edgar 1997).

Studies of *N. tetricus* and *N.fucicola* have concentrated on the adult phases, revealing that they are site attached (Barrett 1995b), and are important generalist carnivores of invertebrates (Denny and Schiel 2001; Shepherd and Clarkson 2001). Both species are also increasingly economically important as the target of a live fishery in south-eastern Australia (Lyle and Hodgson 2001). Although *N. fucicola* and *N. tetricus* are sufficiently closely related that they can form hybrids in the wild (Ayling 1980), they have strongly contrasting reproductive strategies. *Notolabrus tetricus* is typical of most wrasses, being a protogynous hermaphrodite with large, aggressively territorial terminal phase males. It is monandric, with no initial phase males detected in behavioural or histological studies (Barrett 1995a). *Notolabrus fucicola* is a gonochorist, with sexes fixed at maturity, and shows no strong territorial behaviour (Barrett 1995b; Denny and Schiel 2002).

Like the majority of marine demersal fishes, wrasses in the genus *Notolabrus* show a bipartite lifecycle. Juvenile/adult populations associated with the benthic habitat are separated in space and time by a planktonic/pelagic dispersive phase. As *N.*

fucicola and *N. tetricus* are site-attached (Barrett 1995a) the major source of replenishment of individuals to a population arrives from the plankton. Thus, to understand the mechanisms maintaining populations and enabling *Notolabrus* spp. to persist locally, it is necessary to identify the processes influencing the survival of early life stages.

In general, the problem facing dispersive early life stages of any organism are similar, namely dispersing between widely spaced patches of habitat whilst vulnerable to starvation, predation, and adverse transport processes. For *Notolabrus* in the coastal waters of the east coast of Tasmania, high levels of unpredictability in the pre- and post-settlement environment make maximising fitness complex. *Notolabrus fucicola* and *N. tetricus* have a similar, relatively prolonged spawning season, with gonosomatic indices peaking over late winter and spring (Barrett 1995a; Denny and Schiel 2002), although running ripe fish are present in small numbers into January (mid-Summer) in Tasmania. The planktonic environment encountered by *Notolabrus* propagules shows variation in levels of temperature and productivity at a variety of spatial and temporal scales (Harris *et al.* 1987; Harris *et al.* 1988; Harris *et al.* 1991; Harris *et al.* 1992). In some Tasmanian and other temperate fish species, larval production, growth and survival have been correlated with medium scale, daily phenomena such as storms (Thresher *et al.* 1989c), large scale within year phenomena such as increasing day length and temperature (e.g. Jordan 1994; Jordan *et al.* 1995; Garvey *et al.* 2002), and longer term, ocean-wide phenomena such as El Niño and La Niña (e.g. Cowen 1985; Jordan 1994; Jordan *et al.* 1995). Post-settlement *Notolabrus* must also contend with spatial and temporal variability in the productivity and structural complexity of reef habitat. Storms remove kelp, which juvenile fish use for cover (Jones 1984a; Carr 1994), and seasonal cycles of macrophyte production affect

the standing crop of small invertebrates on which many juvenile fishes feed (Jones 1988; Ebeling and Hixon 1991; Edgar 2001). Thus the problem faced by these wrasses is producing larvae that can survive to settle and recruit, while spawning during the period when the pre- and post-settlement environment is highly variable and unpredictable. With protocols for the interpretation of *N. tetricus* and *N. fucicola* otoliths (chapter 3), a method for identifying ambiguous specimens using genetic techniques (chapter 2), description of aspects of the pre-settlement and post-settlement lives of these species, and the interactions between life-history strategies and the strictures of the temperate environment is now possible.

Therefore, the objectives of this study were to 1) reconstruct the distribution of spawning and settlement in recently settled *Notolabrus* spp., 2) determine variability in size at settlement and planktonic larval duration in recently settled *Notolabrus* spp., 3) investigate the effects of pre-settlement parameters on post-settlement growth, and 4) investigate the interactions between environmental correlates, including sea-surface temperatures, on the observed settlement dynamics *Notolabrus* spp.

Materials and Methods

Field sampling

Post-settlement wrasses were collected at three sites on the east coast of Tasmania (Fig. 4.1), over three settlement seasons. Wrasse at Bicheno (41.82°S, 148.30°E) and Recherche Bay (43.59°S, 146.92°E), were collected from rock lobster (*Jasus edwardsii*) puerulus collectors (Gardner *et al.* 1998).

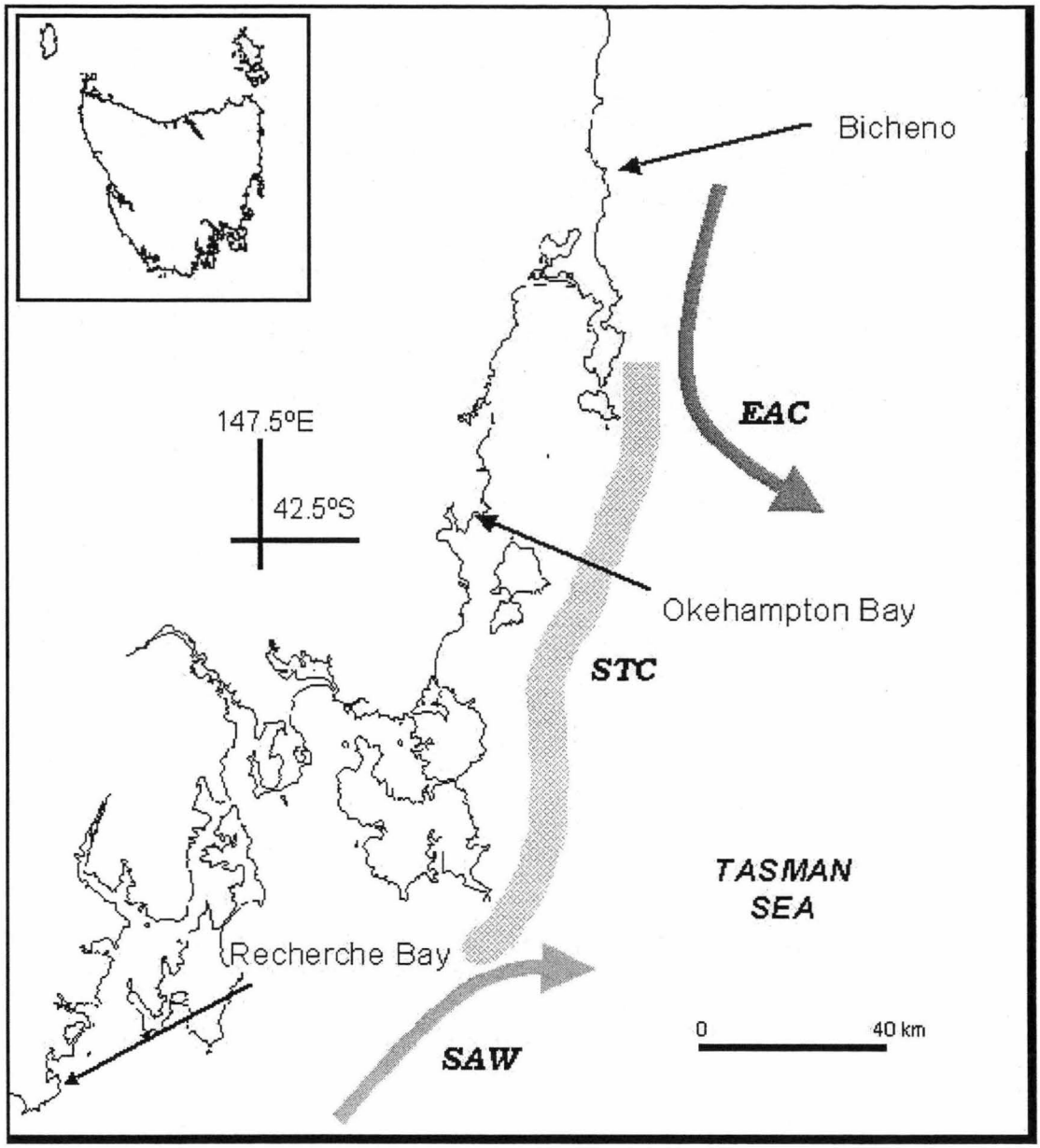

Figure 4.1. Sampling sites where post-settlement wrasses were collected on the east coast of Tasmania. Also shown are the 'typical' extent of the water masses in this region known to strongly influence productivity over Spring Summer and Autumn. (EAC = East Australian Current, SAW = Sub-Antarctic Water, STC = Sub-Tropical Convergence).

Thirty-two and 16 collectors were sampled, at Bicheno and Recherche Bay respectively, approximately monthly during each settlement season. At Okehampton Bay (42.53°S, 147.98°E), wrasse were collected from 20 similar artificial habitats, but designed to be serviced on snorkel. Artificial habitats at Okehampton Bay consisted of

3-5 sheets of corrugated plastic roofing material, bolted together to form crevices, forming a 'head' 0.5×0.5×0.2 m, with a steel tube anchored through the centre of the roofing sheets (Fig. 4.2). The heads were attached via a wire pin to a steel post anchored by a 25 kg concrete block, 0.5 m above the seafloor. Sampling these collectors involved enclosing the collector in a 1 mm mesh bag, pulling the wire pin, and bringing the head to the surface, thereby capturing wrasse that had settled on the habitat. All artificial habitats had been 'conditioned' *in situ* for at least 2 months before sampling began. Bicheno and Recherche Bay were sampled over three settlement seasons (1998-1999 through 2000-2001) and Okehampton Bay was sampled over the 1999-2000 and 2000-2001 seasons (Fig. 4.3).

Fish were euthanased using 2 $g.L^{-1}$ benzocaine in seawater, and preserved in absolute ethanol, and standard lengths (SL - from the anterior tip of the snout to the posterior margin of the caudal peduncle) recorded. Each specimen was identified to the lowest taxonomic level possible using the keys of Russell (1988) and Gomon *et al.* (1994). Sixty-two specimens identified to *Notolabrus*, but of indeterminate species, were identified using mitochondrial DNA random fragment length polymorphisms (Chapter 2).

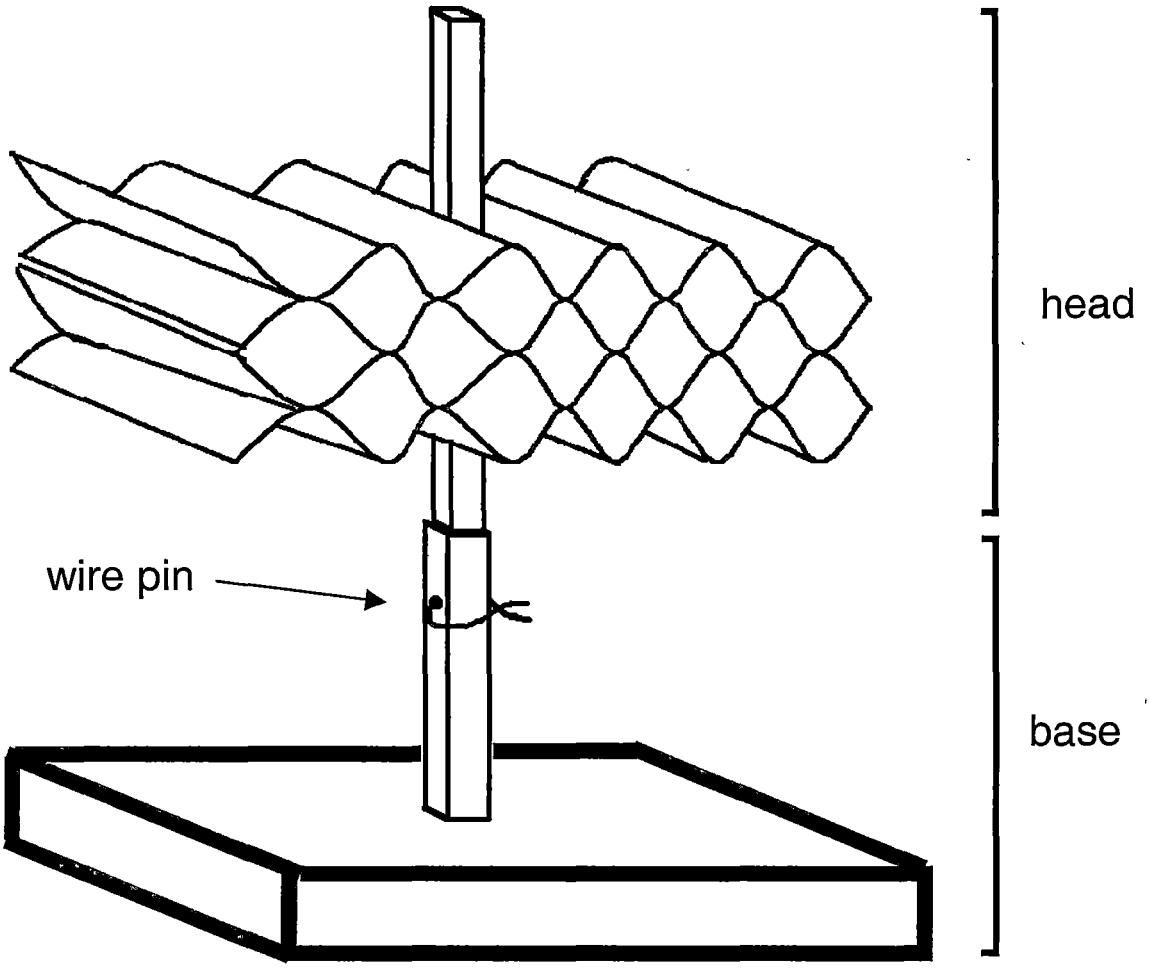

Figure 4.2. Diagram of the artificial habitats used to attract settling wrasses at Okehampton Bay. Not to scale.

Otolith analyses

Preparation

Sagittae and lapillae were extracted under a binocular dissecting microscope, before embedding, sulcal side down, in thermoplastic resin on a glass microscope slide. In the first instance, a randomly selected sagitta from each individual was prepared for interpretation following the procedure described in Chapter 3 (p. 46). In instances where sagittae were unsuitable for analysis, lapillae were used, being prepared in the same way as sagittae.

Measurement and interpretation

Images of otoliths were digitised at ×400 under transmitted light and measured, from the primordium, along the radius of maximum growth to the nearest 0.1 μm using image analysis software.

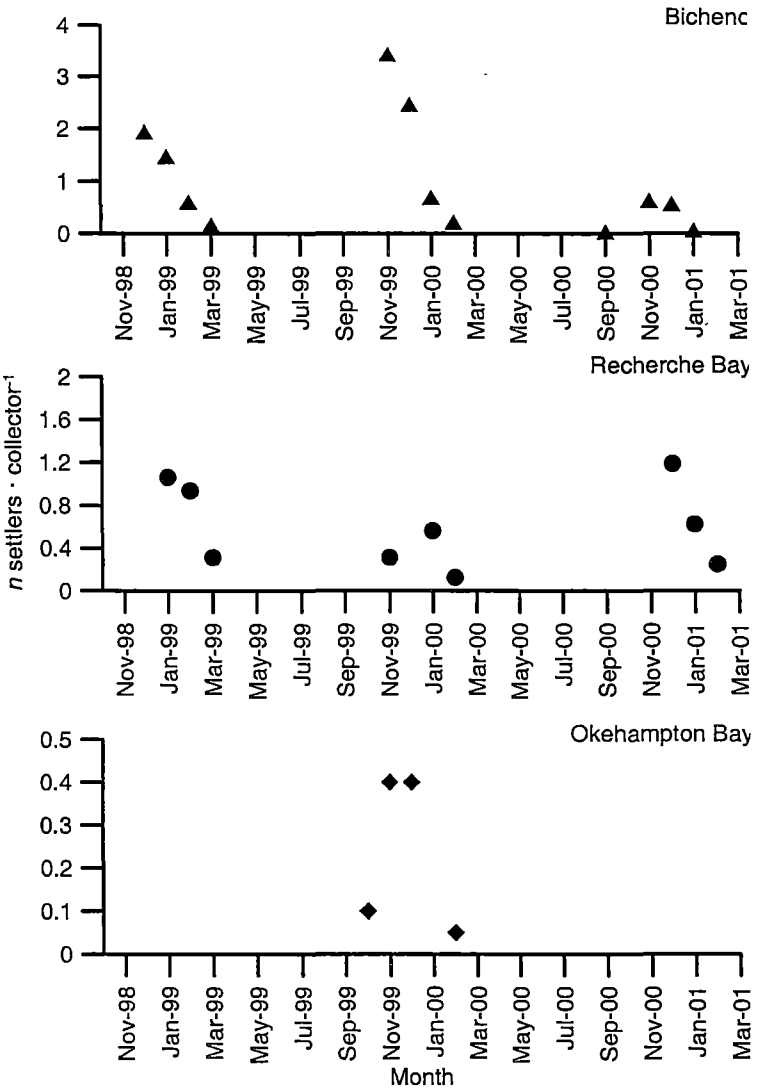

Figure 4.3. Sampling dates and *Notolabrus fucicola* settlers captured per artificial habitat, on the east coast of Tasmania. Okehampton Bay was sampled in 2000-01, but no *N. fucicola* were captured.

For each individual otolith, the number of increments was counted twice or until two counts within a reading session agreed within 4 increments, up to a maximum of four counts. The mean of the two closest counts, rounded to the nearest

integer was then determined as the best estimate for that otolith in that reading session. For sampling dates at Bicheno where more than 50 individuals were captured, a random sample of 50 was selected for otolith analysis.

The repeatability of daily increment counts was tested within and between readers by randomly selecting 51 sagittal otoliths for rereading by the primary author, and 39 otoliths which were reread by an experienced analyst of hard part microstructure[1]. To compare the equivalence of counts between lapillae and sagittae, 25 individuals had both otolith types read blind. Comparisons between counts were conducted by producing age bias plots (Campana *et al.* 1995; Campana 2001), superimposed on a 1:1 correspondence line.

Verification of settlement mark

A clear check mark was evident in the sagittae of approximately 50% of all individuals examined, appearing as an opaque zone darker than in the adjacent increments (Fig. 4.4). Check marks of this nature have been identified in the otoliths of many demersal fishes, and has been shown to be associated with settlement or the process of metamorphosis from planktonic larva to demersal juvenile (Victor 1982; Victor 1986b; Thresher *et al.* 1989b; Wellington and Victor 1989; Jenkins and May 1994; Wilson and McCormick 1999). To determine the relationship between this check mark and settlement (i.e. moving permanently from the planktonic environment to live on the reef) in *N. fucicola*, the distribution of increment counts to the settlement mark was compared with the distribution of counts from individuals that had settled very recently. A specimen was deemed to have settled very recently (in the

[1] Dr Greta Pecl, Research Scientist, Taroona Marine Research Laboratories, Tasmanian Aquaculture and Fisheries Institute, University of Tasmania.

last few days) if it retained larval characters of transparent body with little melanophore pigmentation and bore no scales (Chapter 2).

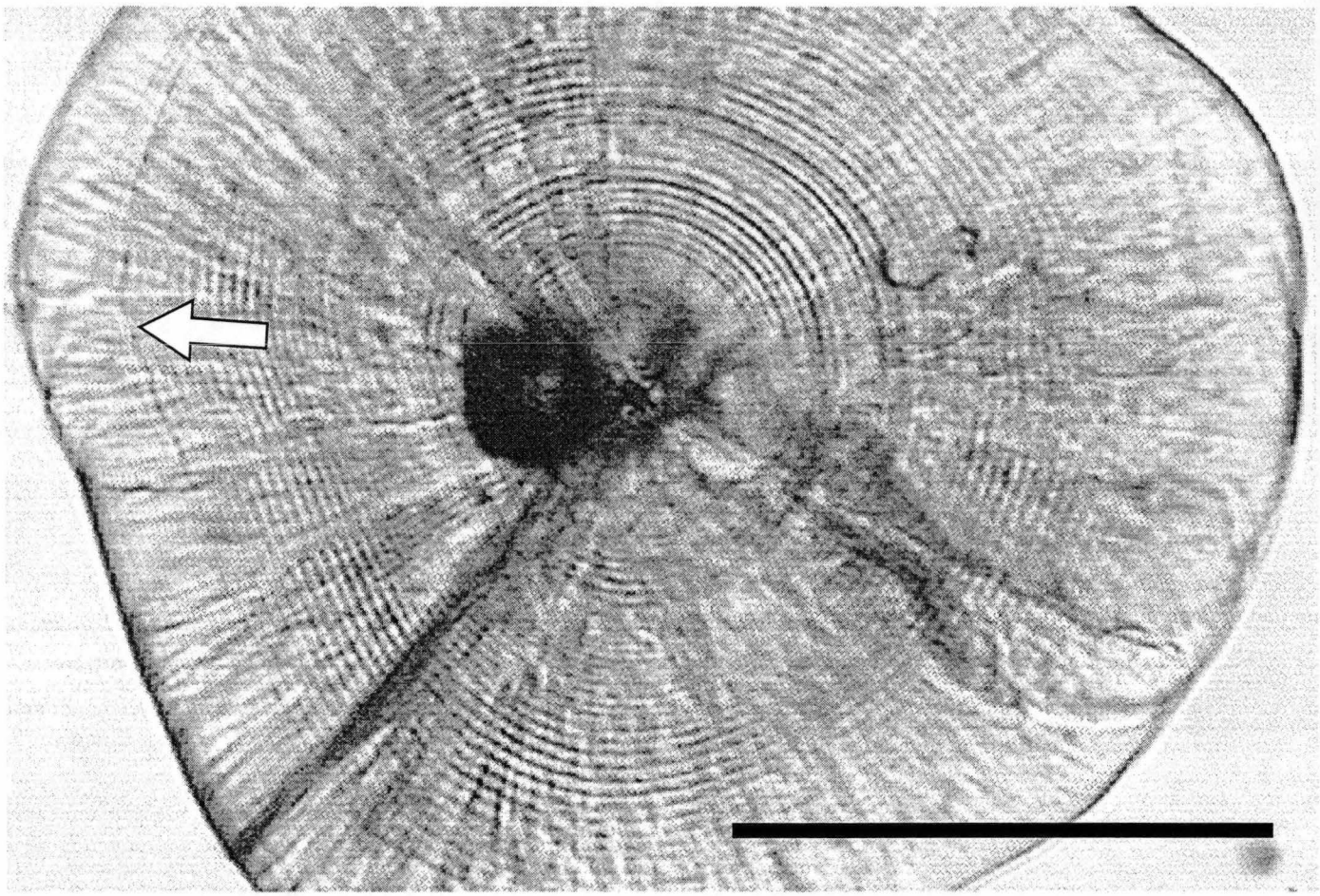

Figure 4.4. Sagitta of a recently settled, 12.4 mm standard length *Notolabrus fucicola.* Arrow indicates settlement mark in the otolith, indicating that this individual settled approximately 9 days before capture, at an estimated total age of 65 days. Scale bar is 200 μm.

Determination of age, larval duration, and spawning and settlement date

To determine the age (days) and planktonic larval duration for each individual, 12 days was added to the total number of increments and the number of increments inside the settlement check respectively. This took into account the time taken for *Notolabrus* spp. to reach first-feeding and begin laying down daily increments in the

sagittae (Chapter 3). Twelve days was chosen rather than 14 as determined in chapter 3, assuming that individuals in this study are likely to have encountered average ambient temperatures higher than the 10°C at which wrasse larvae were successfully reared, and hence would have developed slightly more rapidly. The spawning date was back calculated by subtracting the age from the date of capture, and settlement date calculated by subtracting the number of increments beyond the settlement check from the date of capture. For comparisons of distributions of spawning dates and settlement dates between sites and seasons, all were standardised as the number of days after the 1 July immediately preceding the sampling season.

Patterns of spawning, settlement and growth

The effect of sample year and site on spawning date was tested using ANOVA. The effect of spawning date on planktonic larval duration was analysed between sites and years using ANCOVA, with spawning date as the independent covariate with planktonic larval duration. The relationship between spawning date and size-at-age was analysed on residuals from a linear regression of standard length over total increment count, using ANCOVA with spawning date covarying with regression residuals. In each case, normal quantile plots were used to investigate normality of data, and homogeneity of variances between groups was tested using Bartlett's test. If necessary, the Box-Cox transformation (Sokal and Rohlf 1995) was performed on the response variable to improve the fit to the model assumptions.

To examine for lunar periodicity in spawning or settlement, the distribution of spawning and settlement dates was plotted with moonphases during each settlement season. Autocorrelation analysis was undertaken on proportion of settlement (or spawning) in two day intervals for each yearly sample, with a lag up to the total number of intervals, to test for periodicity at other time scales.

Estimated monthly sea surface temperature (SST) (Halpern *et al.* 2000; Halpern *et al.* 2001; Halpern *et al.* 2002), interpolated to the nearest 1 °C for the inshore waters adjacent to the Recherche Bay and Bicheno sampling sites, was used as a proxy estimate of the temperature of the pelagic environment during the period of settlement to these sites. Spatio-temporal patterns of SST were also used as an indicator of the relative influence of warm, nutrient poor East Australian Current (EAC) and cooler, nutrient rich and subantarctic water (SAW) on the water column off the East coast of Tasmania over the sampling period. Interactions between these water bodies have been shown to influence the timing and duration of the spring plankton bloom off the east coast of Tasmania (Harris *et al.* 1987; Harris *et al.* 1988; Harris *et al.* 1991; Harris *et al.* 1992), with flow on effects to the spatio-temporal dynamics of zooplankton and pelagic fish (e.g. Young and Davis 1992; Young *et al.* 1993; Young *et al.* 1996).

Results

Field sampling

Four species of wrasses (Purple wrasse *Notolabrus fucicola*, blue throat wrasse *N. tetricus*, rosy wrasse *Pseudolabrus psittaculus*, and Castlenau's wrasse, *Dotalabrus aurantiacus* in order of abundance) were captured as recently settled juveniles. Using keys and mDNA RFLP analysis, the great majority of wrasse captured were identified as *N. fucicola*, with *N. tetricus* only constituting a large proportion of recent settlers at Okehampton Bay (Table 4.1).

The artificial habitats used in this study retained a distinct size range of wrasses, from immediately after settlement at a minimum size of 10.4 mm up to

individuals ca. 40 mm SL. A few fish 40 - 50 mm SL were captured, but their scarcity on artificial habitats, and observations of post-settlement *Notolabrus* spp. on a reef nearby the Okehampton Bay site suggests a change in habitat usage at around this size. Above ca. 40 mm, individuals change from being very cryptic and stationary, hiding among macrophytes, to become more vagile, moving around outside the macrophyte canopy as well as within it, similar to the behaviour described for juvenile *N. celidotus* (Jones 1983; Jones 1984a). Vagile fish are less likely to be attracted to and attached to artificial habitats, and so are under-sampled at sizes >40 mm SL.

Table 4.1. Total abundance and species composition of wrasses captured at three sites on the east coast of Tasmania. Counts in each cell are *Notolabrus fucicola* / *N. tetricus* / *Pseudolabrus psittaculus* / *Dotalabrus aurantiacus*.

	Season		
Site	1998-1999	1999-2000	2000-2001
Bicheno	133 / 1 / 3 / -	218 / 1 / 2 / -	41 / 1 / 6 / -
Recherche Bay	53 / - / - / -	16 / - / - / -	33 / 2 / 1 / -
Okehampton Bay	No data	17 / 28 / - / -	- / 7 / - / 1

Otolith analyses

Measurement and interpretation

Regression of maximum sagittal radius against standard length (SL) showed highly significant linear relationships (P<0.01) in both *N. fucicola* and *N. tetricus* (Fig. 4.5). ANCOVA of otolith radius by species with SL as covariate showed a significant interaction effect between species and SL ($F_{1,387}$ =29.8, P= <0.0001), indicating that the slope of the relationship is species-specific. For the range of sizes available, *N.*

tetricus tended to have larger otoliths than *N. fucicola* of equivalent length, however there was too much variability in this relationship to use it as a species-specific diagnostic character. For both species, individuals below approximately 15 mm SL showed smaller otoliths for their length predicted by the regression line. This implies a period of decelerating otolith growth relative to body length during the period immediately after settlement in *Notolabrus* spp.

Plots comparing count estimates of sagittal daily increments within and between readers showed no evidence of bias relative to a 1:1 correspondence line (Fig. 4.6 a, b). For individuals from which lapillar and sagittal counts were available, estimates were equivalent between otolith types (Fig. 4.7). Consequently otolith increment counts by the primary author were considered consistent, and were used to determine age estimates and back-calculations of spawning and settlements dates.

Verification of settlement mark

Based on their retention of larval characters, 26 *N. fucicola,* across all sites and years, were identified as having settled within a few days of capture. Box plot comparisons of sagittal increments out to the transition mark with counts in the sagittae of these post-larvae showed close correspondence in the two distributions (Fig. 4.8). A *T*-test assuming unequal variances showed no significant difference between the two groups (T_{34} = 1.94, P= 0.06). Therefore the transition in the sagittae was considered to mark the day of settlement, and was used in estimating planktonic larval duration (PLD), and back-calculated settlement dates for individual fish.

Age at settlement was highly variable in *N. fucicola*, with PLD varying between 40 and 87 days (i.e. 28-75 pre-settlement increments + 12 to account for days to first increment forming), with a median value of 61 days over all samples. Size at settlement was also variable, as evidenced by the spread of sizes in individuals that

had recently settled (Fig. 4.9). Few recently settled *N. tetricus* were captured, but they showed a slightly shorter PLDs and a narrower range (44-66 days, median 54 days) than *N. fucicola*, and a slightly larger size at settlement (Fig. 4.9). This suggests that *N. tetricus* may grow faster than *N. fucicola* through the pre-settlement phase.

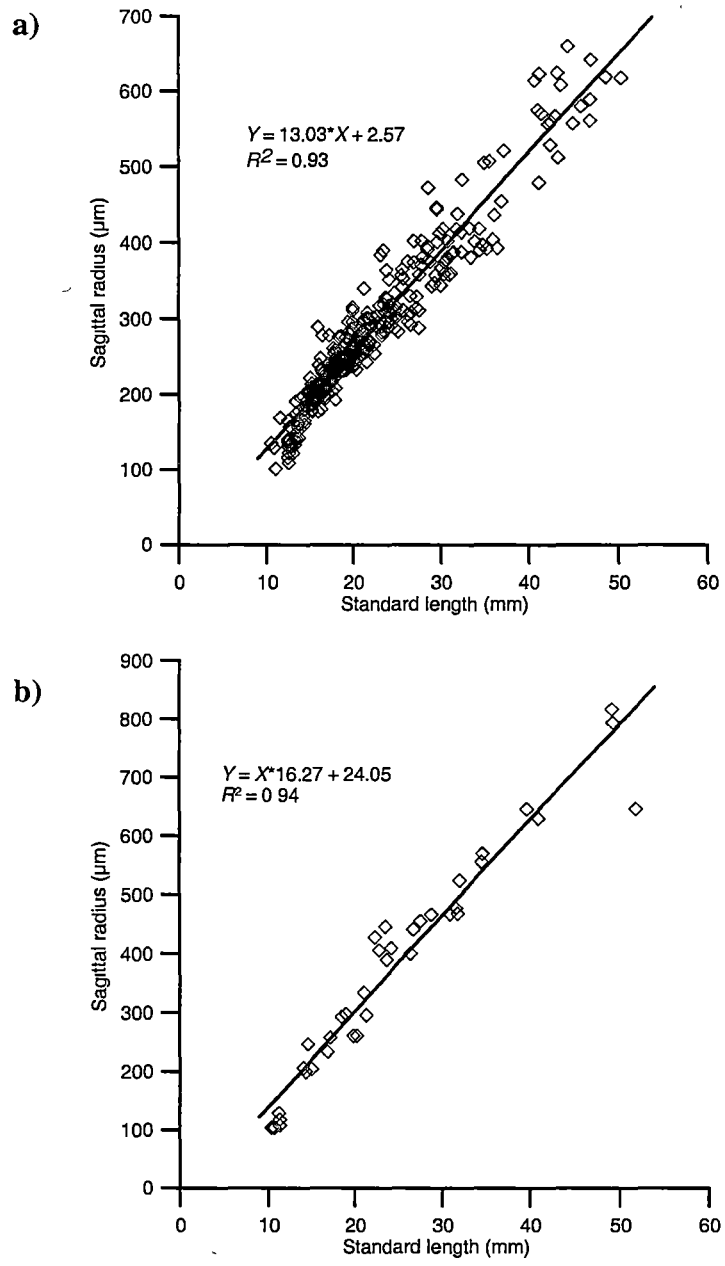

Figure 4.5. Plots of maximum sagittal otolith radius versus standard length in post-settlement wrasse, pooled across all fish collected over 1998-2001. **a)** *N. fucicola* ($N = 354$) **b)** *N. tetricus* ($N = 37$).

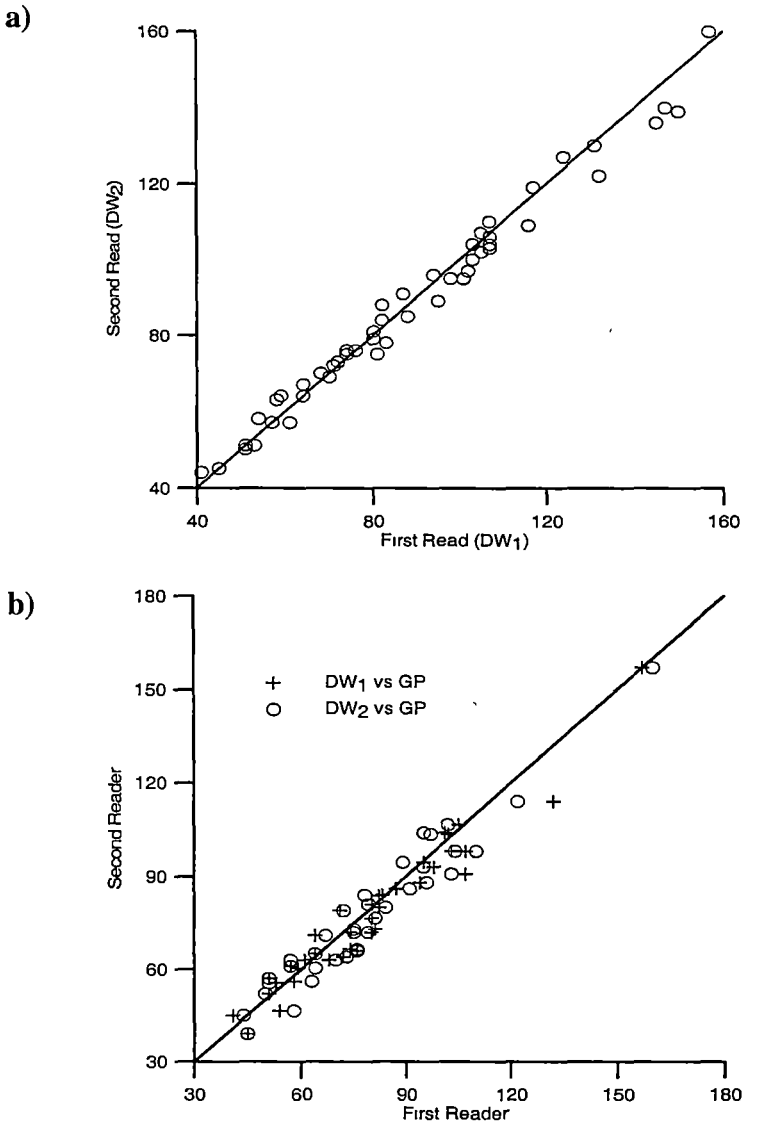

Figure 4.6. Precision of increment counts of post-settlement *Notolabrus* spp. **a)** Repeat counts by the primary reader (N=51) **b)** Repeat counts by independent readers (DW_1 vs GP, N= 38, DW_2 vs GP, N= 39). In both plots, the 1:1 correspondence line is shown.

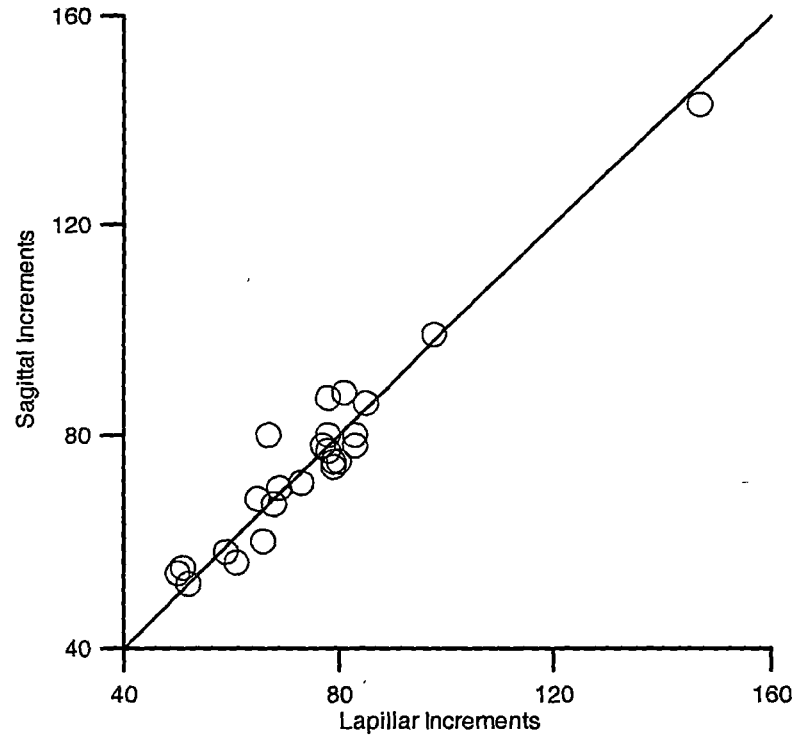

Figure 4.7. Increment counts of lapillar and sagittal otoliths from the same individuals (*N*=25). 1:1 correspondence line is shown.

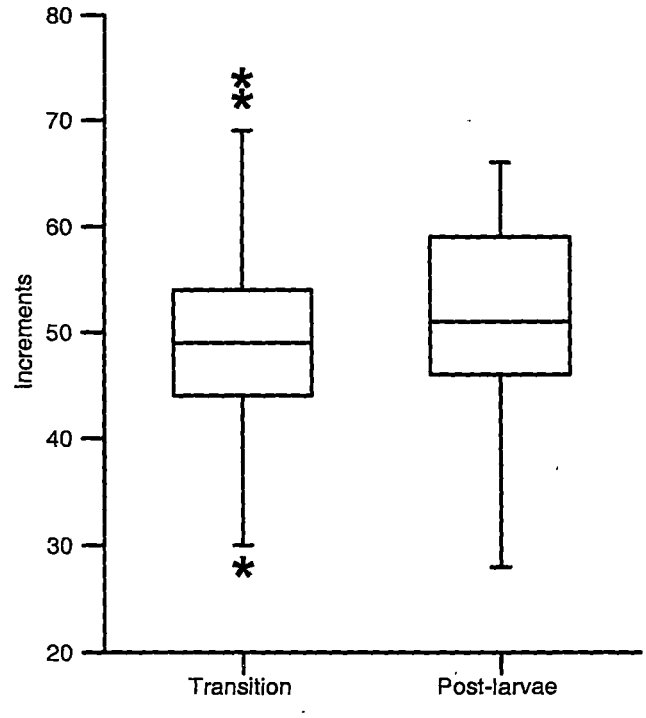

Figure 4.8. Box plots of increment counts to the transition mark in the sagittae of 186 post-settlement *Notolabrus fucicola* (Transition), compared to the total increment counts in the sagittae of 26 recently settled *N. fucicola* still showing larval morphology (Post-larvae). Outliers indicated by *.

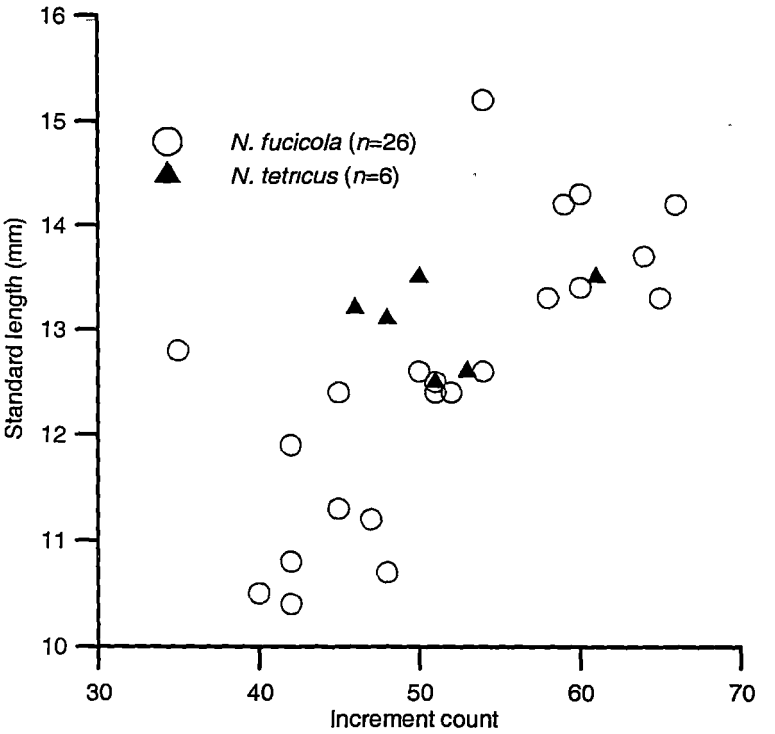

Figure 4.9. Variation in size and increment count for wrasses, deemed to have settled recently (based on retention of larval morphology and pigmentation).

Patterns of spawning , settlement and growth

Lunar periodicity

Because 1998-99 and 1999-2000 settlement seasons at Bicheno produced the most specimens, these years were inspected in the first instance for evidence of any lunar periodicity in back-calculated spawning and settlement dates. Within the 1998-99 season, the largest peak of spawning dates occurred between the new and full moon (Fig. 4.10 a), resulting in a peak of settlement around the new and full moon two months later (Fig. 4.10 c). Peaks corresponding to the new and full moon occurred in both spawning and settlement during the 1999-2000 season (Figs. 4.10 b, d). However, there was no strong relationship between lunar cycle and the proportion of annual spawning or settlement dates, and autocorrelation plots revealed no strong periodic signals in settlement or spawning.

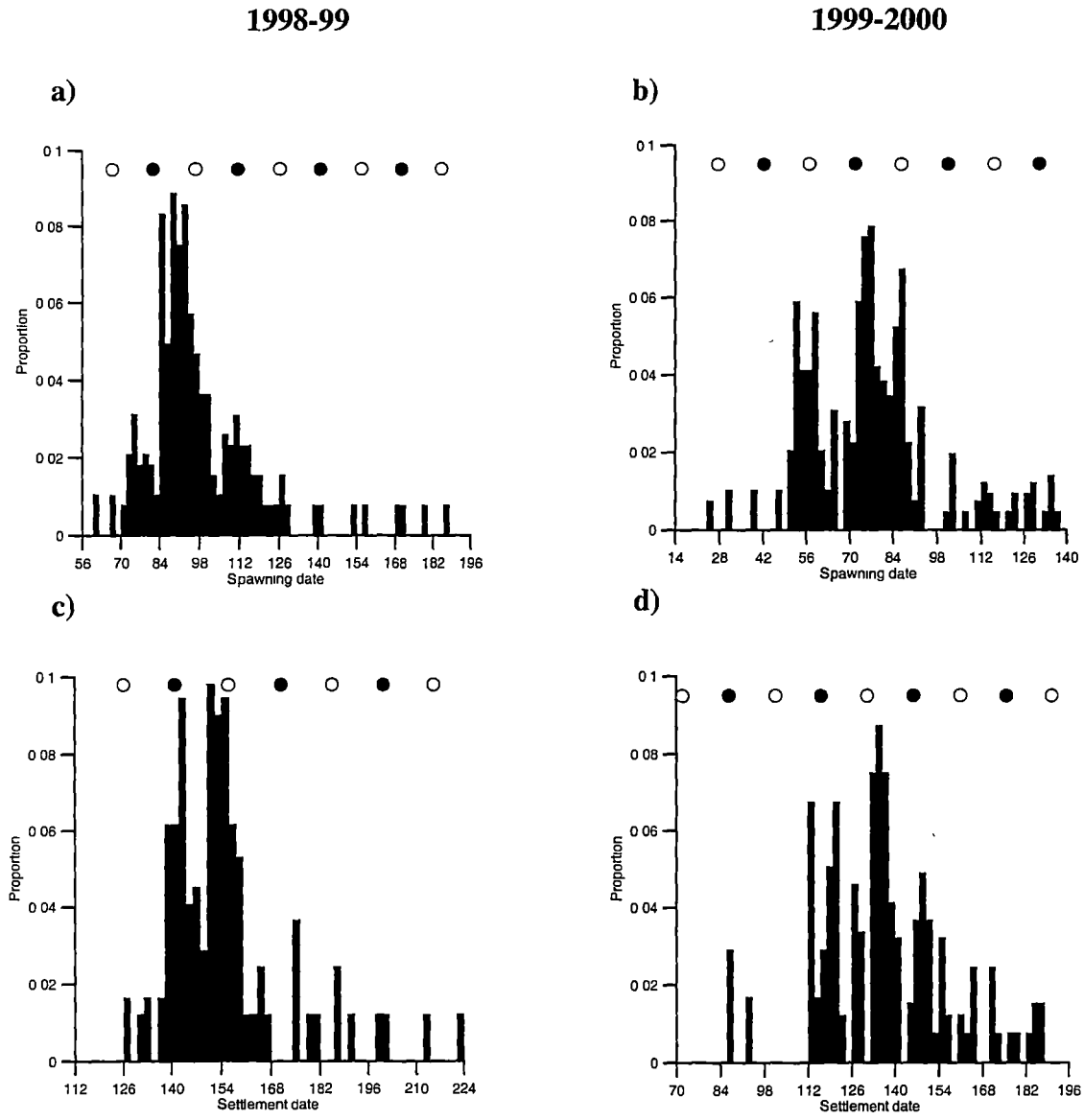

Figure 4.10. Distributions of back-calculated spawning dates, **a)** 1998-99, **b)** 1999-2000, and settlement dates, **c)** 1998-99, **d)** 1999-2000, of *N. fucicola* at Bicheno. Settlement date is arbitrarily depicted as days since 1 July each year preceding the settlement season.● = new moon, ○ = full moon.

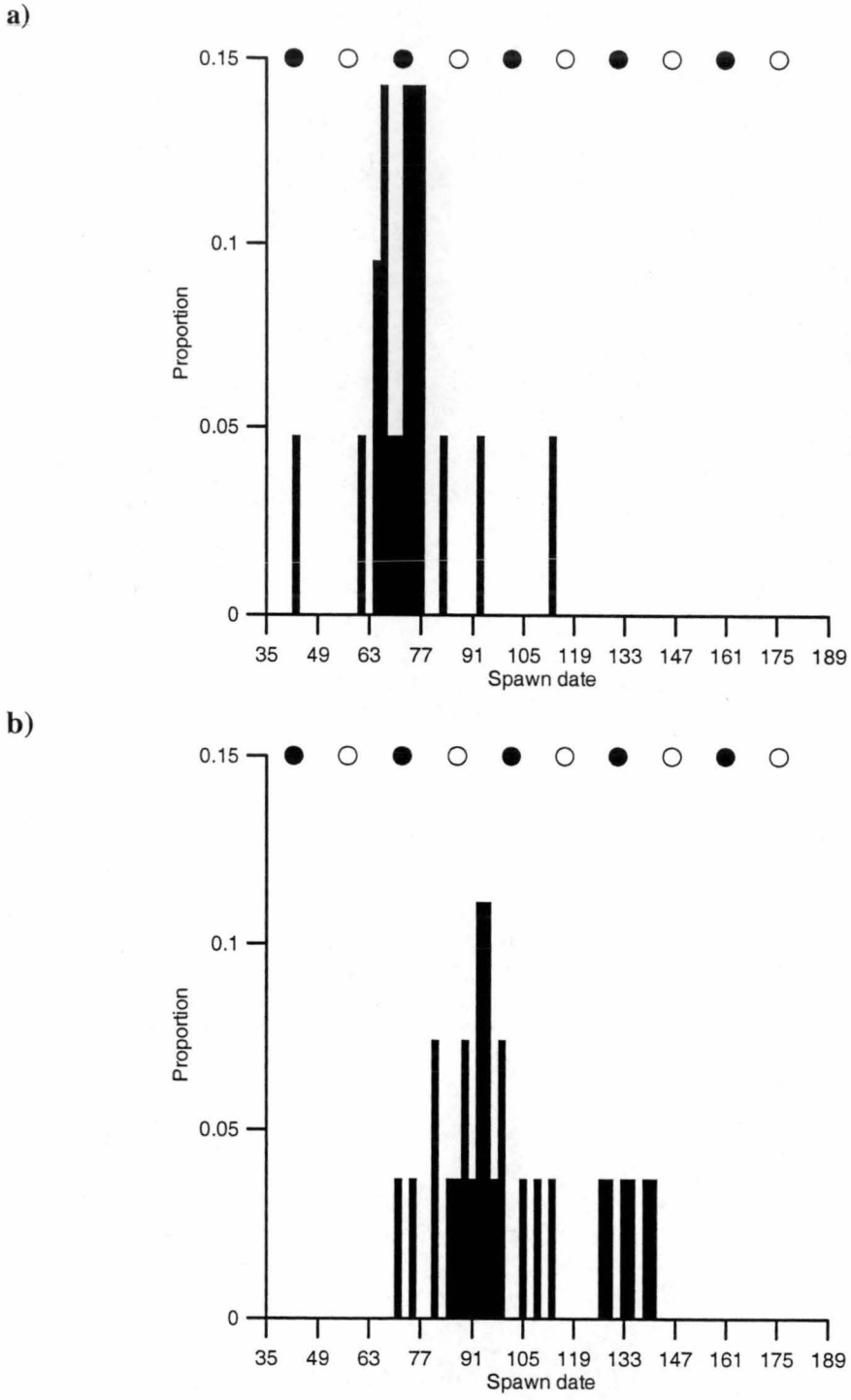

Figure 4.11. Spawning date distributions of **a)** *Notolabrus fucicola* and **b)** *N. tetricus* at Okehampton Bay, 1999-2000. Settlement date is arbitrarily depicted as days since 1 July 1999. ● = new moon ○ = full moon.

Species-specific spawning patterns

Settlement at Okehampton Bay during the 1999-2000 season was the only sample to produce both *N. fucicola* and *N. tetricus* in comparable numbers (Table 4.1). Analysis of this sample indicated that *N. tetricus* settlers were spawned later in the season than *N. fucicola* (Fig. 4.11). Spawning in the two species overlapped, but the major peak in spawning that produced settlers was at least 14 days earlier in *N. fucicola*.

Spatial and temporal spawning patterns

Both site and year significantly and independently influenced spawning dates of *N. fucicola* at Bicheno and Recherche Bay (Table 4.2). Box plots of spawning dates show consistently later spawning dates for settlers at Recherche Bay within all three years (Fig. 4.12). *Post-hoc* comparisons of mean spawning dates indicated that settlement at Recherche Bay occurred 26.8 (± 4.4 S.E.) days after Bicheno. Pair-wise comparisons of mean spawning dates across years indicated that spawning occurred significantly later in 1998-99 than both 1999-2000 (25.3 ± 4.0 S.E. days) and 2000-01 (24.7 ± 4.9 S.E. days), while the distribution of spawning in the latter two years was not significantly different at either site.

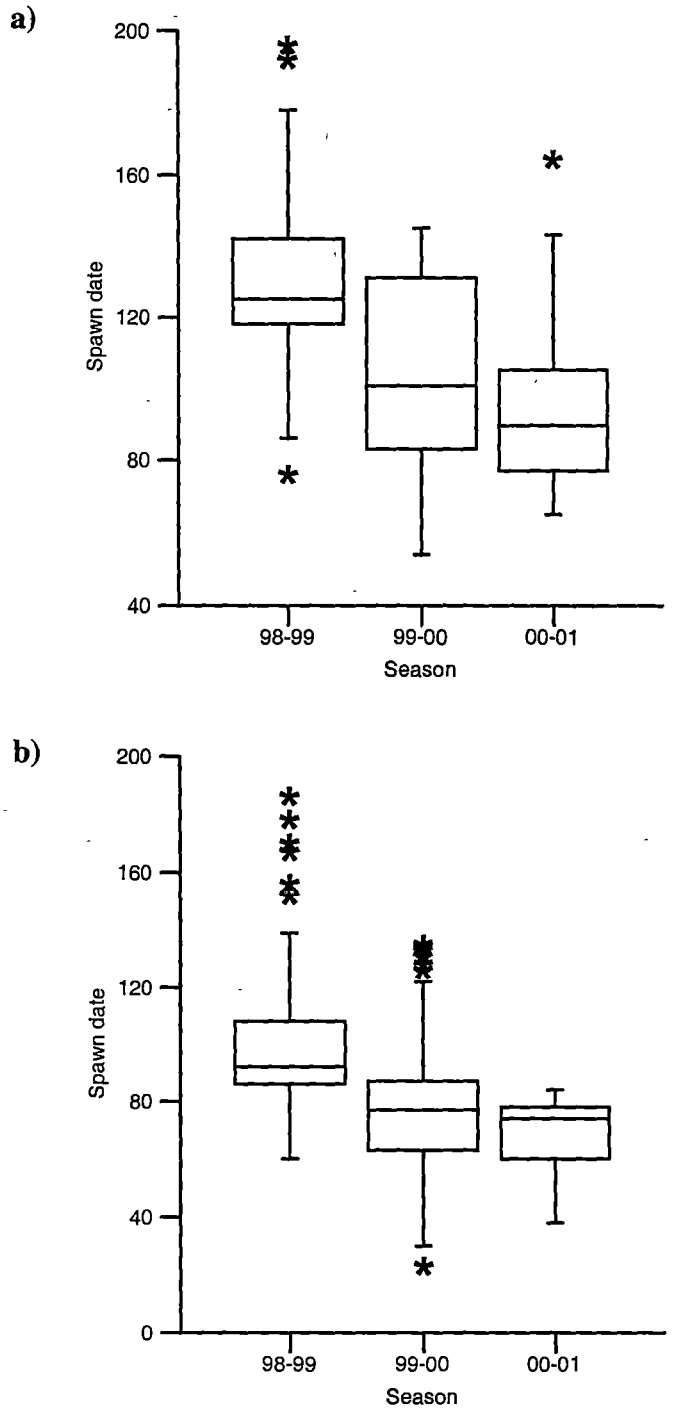

Figure 4.12 Box plots of back calculated spawning date distribution of all post-settlement *N. fucicola*, **a)** Recherche Bay and **b)** Bicheno1998-2001. Spawning date is days since 1 July in each year. *=outliers

Table 4.2. Results of ANOVA for effects of sampling site (Recherche Bay and Bicheno), and settlement year on spawn date of *Notolabrus fucicola.*

Source	Sum of Squares	df	Mean Square	*F* ratio	*P*
Site(S)	36977.35	1	36977.35	73.19	<0.0001
Year(Y)	43827.21	2	21913.61	43.37	<0.0001
S*Y	768.09	2	384.05	0.76	0.468
Error	178332.96	353	505.2		

The peak of back calculated spawning dates tracked the pattern of monthly mean SSTs offshore of Bicheno and Recherche Bay (Halpern *et al.* 2000; Halpern *et al.* 2001; Halpern *et al.* 2002). Water temperatures adjacent to Bicheno were consistently 2°C higher at Bicheno than Recherche Bay throughout the sampling season in all years. The 17-19° C isotherm was positioned south of Bicheno in December in 1999 and 2000, while offshore temperatures were less than 17 °C at the same time in 1998. No year during this study was classified as El Niño or La Niña, with no evidence of anomalous influence of EAC or SAW water inshore detectable from SST satellite images of (Halpern *et al.* 2000; Halpern *et al.* 2001; Halpern *et al.* 2002).

Relationship between spawning and PLD

ANCOVA of PLD, with spawning date as the independent covariate showed a highly significant effect of spawning date ($F_{1,198}$=54.91, P<0.0001), independent of site or season. Plots of spawning dates against planktonic larval duration (PLD) show a decrease in time spent in the plankton the later individuals are spawned (Fig. 4.13).

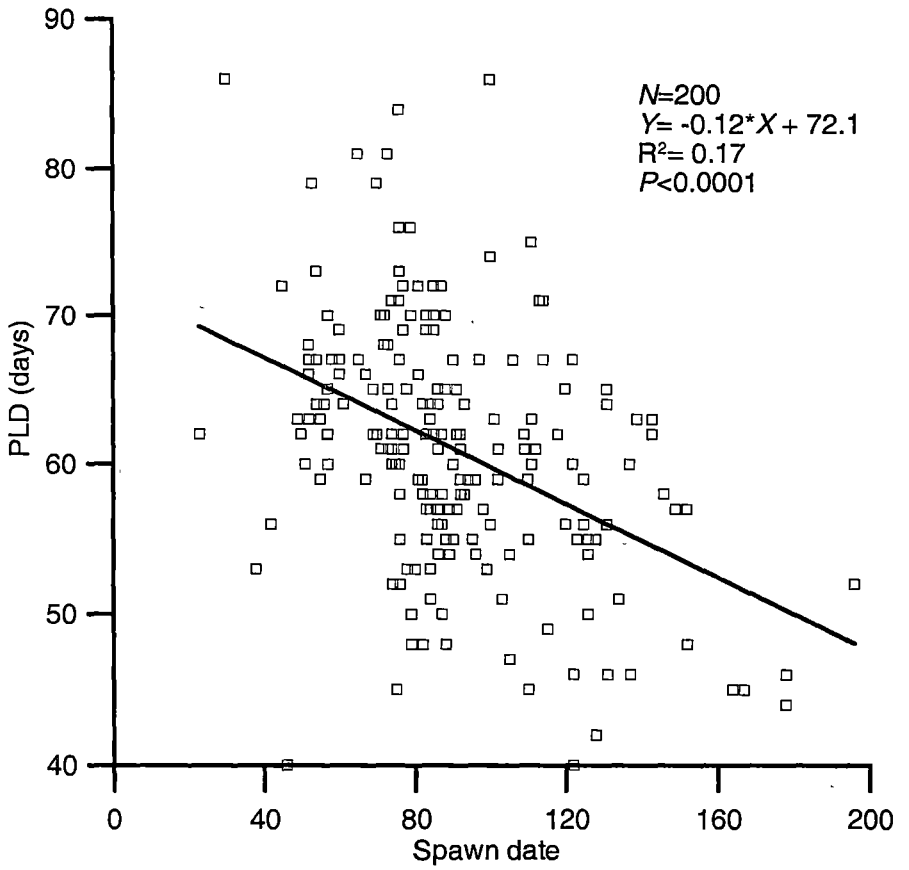

Figure 4.13. Effect of *Notolabrus fucicola* back-calculated spawning date on planktonic larval duration (PLD) across three sampling seasons and two sites. Spawning date is days from 1 July preceding each settlement season.

Post-settlement growth

For *N. fucicola*, total daily increment count was regressed against standard length at capture, including only individuals <40 mm SL to remove the influence of the few large individuals in the dataset (Fig. 4.14). The residuals from this regression were then tested by ANCOVA, across all samples from Bicheno and Recherche Bay with spawning date as a covariate. This test indicated no significant effect on size-at-age of site or settlement date, but a highly significant effect of spawning date on growth residuals ($F_{1,333}$=25.7, P<0.0001). Residuals plotted against spawning date showed individuals spawned later were more likely to have above average size-at-age (Fig. 4.15).

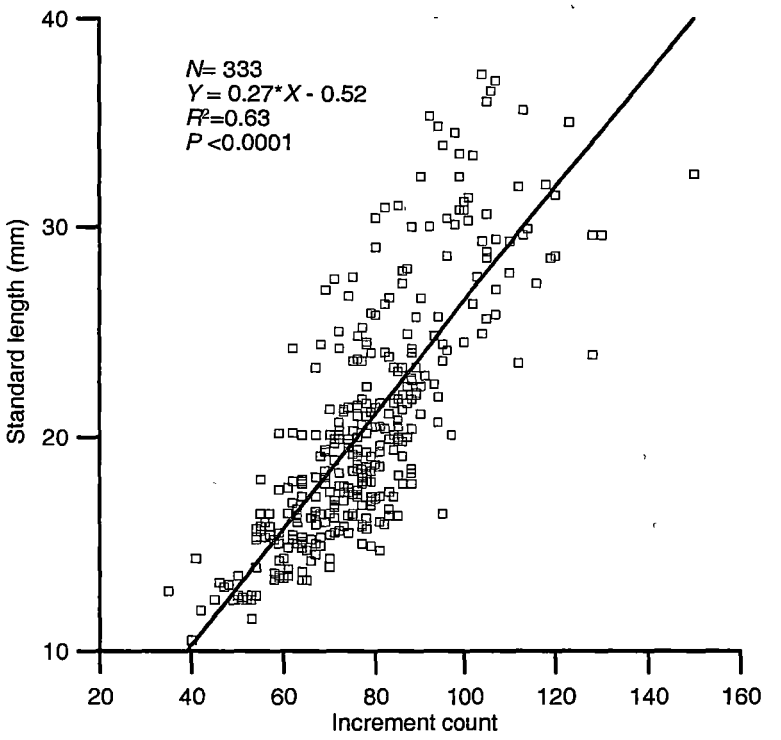

Figure 4.14. Linear regression of otolith increment count against standard length of all *N. fucicola*, pooled across Bicheno and Recherche Bay over three seasons.

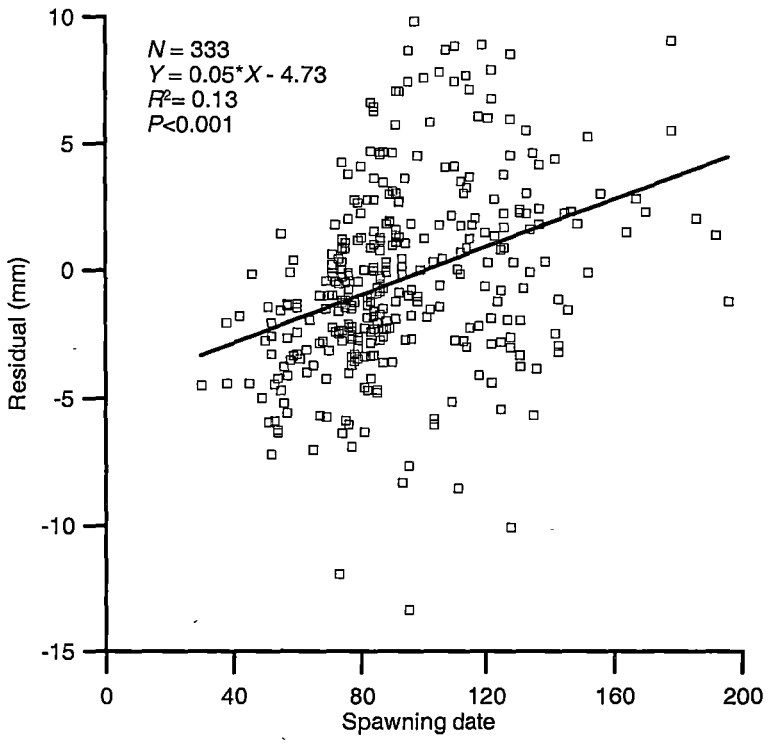

Figure 4.15. Effect of spawning date on residuals derived from Fig. 4.14.

Discussion

In this study, large samples of wrasses were captured using artificial habitats, and validated otolith microstructure was successfully used to determine age and date determine the timing of events in individual wrasses lives. As a result, the pre- and post-settlement lives of southern temperate wrasses can be described in detail for the first time, and inferences as to the interactions between the environment and the life-history strategies of these temperate reef fishes can be made.

Otolith morphology and microstructure

Otolith morphology has been used to identify the larval and post-settlement stages of tropical wrasses (Victor 1987). *Notolabrus* spp., like many labrids, are morphologically conservative, making their identification technically difficult, particularly in the early life-history stages (Chapter 2). A species-specific pattern of otolith growth could provide a rapid method of identifying recently settled wrasses. However, despite a significant species difference in the mean otolith size to body size relationship in this study (Fig. 4.5), individual variability is high, such that any one individual is unlikely to be identified solely based on this relationship.

There is evidence, for the smallest *Notolabrus* spp. individuals, that an allometric change in the size of otoliths relative to body length occurs during the transition from post-larval to juvenile (Fig. 4.5). This change, showing reduced relative growth of the sagittae after the transition point, is concurrent with the final ossification of fin elements and completion of body squamation in individuals between settlement and ~15.0 mm SL (Chapter 2). *Notolabrus fucicola* and *N. tetricus*, like many wrasses and reef associated fishes from tropical coral reefs (Victor 1986b; Victor 1991; Wilson and McCormick 1999), show a transition or mark in their otoliths associated with settlement from the pelagic environment to one associated

with the demersal habitat (Figs 4.3 and 4.7), and this structure is likely to be linked to these morphological and metabolic changes. The departure from linearity in the otolith-body size relationship at small sizes indicates the need for caution in interpreting the otolith record as a direct reflection of the growth record of each individual (Francis 1990; Milicich and Choat 1992). However, the relationships between body size and otolith size and age are correlated such that more detailed analyses of the individual history of growth pre- and post-settlement would be possible, particularly if more information on larval otolith and somatic growth was available. This type of analysis may determine whether the variability in PLD seen in *Notolabrus* is the product of delayed metamorphosis (Victor 1986a; Cowen 1991; Jenkins and May 1994; McCormick 1999), and/or large variations in growth rates between individuals in the plankton, and also increase the rate of detection of increment changes associated with settlement marks, which in this study were only recorded in the most obvious examples (e.g. Fig. 4.4).

Variability in planktonic larval duration

In general, labrids show a high level of intra- and interspecific variation in estimates of PLD when compared with other fish families for which similar multi-species datasets are available e.g. pomacentrids (Victor 1986b; Wellington and Victor 1989; Thresher *et al.*; 1989b). The range of values of PLD seen in *N. fucicola*, from 40 to 87 days post-spawning, places it at the upper end of the variability and maximum PLD observed for other labrids, with only species in the tropical genera *Thalassoma*, *Novaculichthys* and *Xyrichtys* having longer PLDs (Victor 1986b). The few specimens available in this study indicates that a similar pattern of variability is likely for *N. tetricus* (Fig. 4.9).

N. fucicola has a similar range of PLD to the Californian reef wrasse *Semicossyphus pulcher*, with a PLD range of 37 to 78 days (Cowen 1991). This contrasts with the relatively short PLD of the two other relatively well studied temperate (north Atlantic) wrasses, *Tautoga onitis*, with a PLD of 20-30 days (Victor 1986b; Sogard *et al.* 1992), and *Tautoglabrus adspersus*, with a PLD of 18- >37 days (Victor 1986b; Levin 1996). However, the three northern hemisphere wrasses are similar to *N. fucicola* in that they spawn for several months each year. *Tautoglabrus adspersus* and *Tautoga onitis* spawn for 3-4 months over summer and early autumn (Fritzsche 1978; Sogard *et al.* 1992; Levin 1996), and larvae of *S. pulcher* are most abundant in the plankton in mid to late summer (Cowen 1991).

These parallels in life histories of temperate zone wrasses may indicate that similar selection pressures are operating on these species in the different hemispheres. The highly variable PLD in *S. pulcher* may reflect the beneficial ability to delay metamorphosis, enabling a competent wrasse to slow growth and continue to survive in the plankton, whilst waiting for unpredictable onshore transport processes and/or patchy habitat to become available for settlement (Cowen 1991). This hypothesis also applies to *N. fucicola*, as the ability to vary PLD and settle at a variety of sizes (Fig. 4.9) would facilitate survival to settlement under planktonic conditions of slow or unpredictable growth due to low water temperatures and patchy food.

Lunar periodicity in spawning and settlement dates

Many coral reef species show lunar periodicity in their patterns of settlement, stemming from lunar periodicity in production of eggs (Robertson *et al.* 1988), and/or as a product of planktonic processes such as tidally assisted transport (Sponaugle and Cowen 1996; Sponaugle and Cowen 1997) and reduction of visual predation on dark, new moon nights (reviewed by Doherty 1991). However, other environmental

variables such as wind stress and current speeds, and the specific biology of each species have been shown to explain much of the variability seen in availability of larvae inshore, and patterns of settlement in both temperate (Cowen 1985; Thresher *et al.* 1989c; Jenkins *et al.* 1997) and tropical demersal species (Kingsford and Finn 1997; Robertson *et al.* 1999).

The results from this study are equivocal regarding the influence of lunar periodicity on spawning and settlement, with peaks of spawning and settlement occurring near the new and full moons (Fig. 4.10). This suggests that there may be some component of these events that is synchronised with or amplified by the lunar cycle and associated physical phenomena such as tidal currents. However, spawning and settlement also occurred throughout the lunar month in all seasons in this study. Based on observations at Okehampton Bay in 1999-2000, there may also be species-specific patterns of spawning and settlement (Fig. 4.11). Although it is reported that the broad spawning season of *N. tetricus* and *N. fucicola* are the same in Tasmania (Barrett 1995a), asynchrony in spawning activity would serve to isolate spawning populations of *N. tetricus* and *N. fucicola*, and minimise the chances of the hybrids reported by Ayling (1980). However, little information exists on the patterns of spawning of *N. tetricus* or *N. fucicola* between locations, or within spawning populations throughout the spawning season.

The influence of water temperature on settlement dynamics

The results of this study of temperate wrasses suggests that variations in the water temperature on the coast of Tasmania are correlated with the spawning and settlement patterns observed at several spatial and temporal scales. Spawning dates of successfully settling *N. fucicola* within years were consistently later at Recherche Bay relative to Bicheno through all settlement seasons studied, and water temperatures

were consistently lower at Recherche Bay. Similarly, settlement at each site was earlier during years of higher offshore temperatures in spring and summer months (1999-2000 and 2000-01) than when temperatures where ca. 2 °C lower, as occurred throughout the 1998-99 settlement season (Fig. 4.12).

Benefits of later spawning dates

Variability in the distribution of spawning dates had important implications for the pre- and post-settlement lives of wrasses spawned into the coastal waters of Tasmania. In the pre-settlement phase, the time spent in the plankton, though variable, decreased for individuals spawned later in the year (Fig. 4.13). Late spawned larvae were, on average, in the plankton for one week less than individuals spawned two months earlier. For a species such as *N. fucicola* that can spawn for six months each year throughout its range (Barrett 1995a; Denny and Schiel 2002), this produces large variation in the pre-settlement experience of individuals within a year class.

The effect of spawning time also effects post-settlement performance, with the growth rate of later spawned individuals greater than that of their earlier spawned cohort members (Fig. 4.15). Again, the size of this effect is biologically significant. The mean monthly growth rate of post-settlement *N. fucicola* is 8.1 mm overall (Fig. 4.14), but the mean length difference between fish of the same age is 1.5 mm for each month difference in their spawning date (Fig. 4.15). This post-settlement growth relationship is independent of site or year effects, which suggests that compared to the planktonic environment, the effect of the post-settlement habitat is much less variable on early growth of *Notolabrus* than is spawning date. As a result, individuals spawned later in the year may be more likely to survive the planktonic larval phase, because they spend less time in this unpredictable environment, and are therefore less susceptible to mortality due to predation, starvation, or advection away from suitable

habitat for settlement. Later spawned fish also grow at a faster rate than those spawned (and settled) earlier.

Benefits of earlier spawning dates

Two other factors, one operating in the pre-settlement phase, and one operating post-settlement may be contributing to the maintenance of a prolonged spawning season that begins early in the year. Although higher water temperatures mean higher potential growth rates, they also may indicate the intrusion of nutrient poor waters onto the shelf, developing as summer arrives and nutrients are depleted. Alternatively higher water temperatures can indicate the influence of La Niña, when a lack of westerly winds fails to drive local upwelling and the northerly flow of subantarctic water, so that the shelf is dominated by east Australian current waters, and the spring bloom is earlier but shorter than usual (Harris *et al.* 1987; Harris *et al.* 1988; Harris *et al.* 1991; Harris *et al.* 1992). Furthermore, in conditions of water column stratification, resulting from low westerly wind stress, secondary plankton productivity is dominated by small copepod species and heterotrophic microbes, so the majority of plankton biomass is unavailable to higher trophic levels, including fish larvae (Harris *et al.* 1991). In these situations, fish spawned early may be more likely to settle, due to the lack of larval food available later in the season. The converse situation occurs in El niño years, when strong westerlies push warm nutrient poor water offshore, driving upwelling of nutrient rich waters inshore, and advecting subantarctic waters north along the east coast, and the plankton may bloom for longer than usual (Harris *et al.* 1987; Harris *et al.* 1988; Harris *et al.* 1991; Harris *et al.* 1992). This could conceivably result in an unusually prolonged settlement season for *Notolabrus*, due to available food for larvae, or delay settlement as water temperatures stay low and water movement prevents competent post-larvae reaching shore. Neither

of these extreme situations prevailed during this study, so such extrapolation is speculative. Nonetheless, if both are reasonable scenarios, they would serve to maintain selective advantage for individuals able to spawn for an extended period, with the constraints being that in winter water is too cold for plankton or larvae, and that by late summer the bloom will have depleted nutrients, and the food webs reliant on them.

Post-settlement, the reef appears to be a more reliable habitat over spring and summer, as evidenced by lack of significant variation in growth rates between sites and years in this study. However, growth in adult *N. fucicola* shows a dramatic slowing over the coldest months (Chapter 5), which reflect a reduction in productivity in the lower trophic levels on which they feed (Jones 1988; Ebeling and Hixon 1991; Edgar 2001), and reduced activity levels associated with lower water temperatures (Denny and Schiel 2001). Thus, despite growth rate advantages conferred by later settlement, the earlier a settler reaches the benthic habitat, the longer the period of growth before reduced food supplies and temperatures impact on growth potential (Garvey *et al.* 2002). Moreover, size is likely to be advantageous in terms of reduced mortality over winter and beyond (Sogard 1997). The advantage of size is also reinforced by size-related social status in hierarchies common in wrasses, e.g. larger female *N. celidotus* aggressively restrict spawning opportunities of smaller females in the early part of the spawning season on reefs in northern New Zealand (Jones and Thompson 1980). Again, there are advantages to settling and spawning earlier. If size is critical in the post-settlement survival and fitness, timing is everything for temperate reef fishes to ensure that offspring settle as early as possible, as they will potentially achieve a size advantage which can never be overtaken by later settlers. This is illustrated in the case of *N. fucicola* with a simple model, incorporating the

effects of spawning date on planktonic larval duration and post-settlement growth, as determined in this study (Fig. 4.16). Assuming a mean size at settlement of 12 mm, it is evident that even with less time spent in the plankton, and increased growth post-settlement, at any time during the settlement season, earlier spawned individuals retain a size advantage over later settlers.

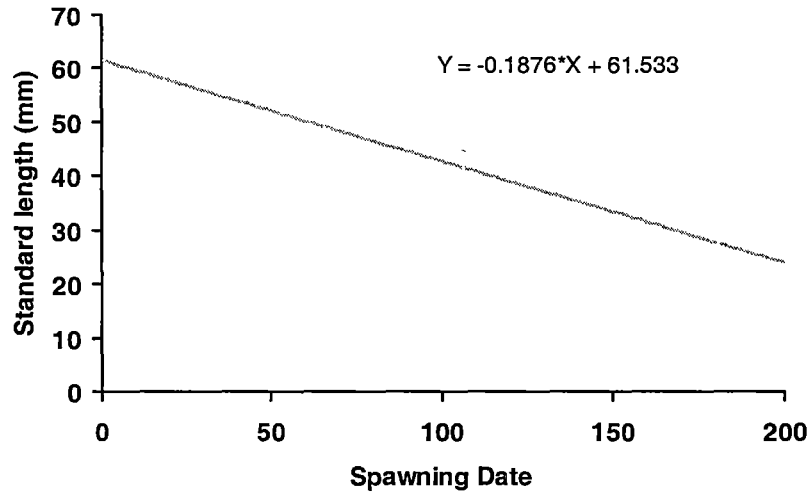

Figure 4.16. Effect of spawning date on expected mean length of *N. fucicola* at day 250 after spawning date 0. The model is based on the relationship between spawning date and larval duration post-settlement growth, as depicted in figs. 4.12 and 4.14. The model assumes mean size at settlement is 12 mm SL, independent of larval duration or spawning date.

Recent research on the advanced abilities of post-larval fishes to sense and orient to potential reef habitat from long distances (Leis *et al.* 1996; Tolimieri *et al.* 2000), and in many cases swim strongly (Stobutzki and Bellwood 1997), indicates that larvae are able to actively influence the circumstances of their settlement based on individual assessment of environmental cues. As suggested by Kingsford and Finn (1997), greater resolution in patterns of adult spawning, pre-settlement behaviour and swimming abilities, along with biological constraints such as time to competency, are required to adequately explain patterns of settlement in reef fishes. Few of these

parameters have been adequately quantified to refine explanatory models of the patterns seen in *Notolabrus*. In particular, nothing is known of the pre-settlement distribution or behaviour of wrasse larvae in Tasmania, and the transport mechanisms moving larvae from their spawning location and to the sites of their settlement. Further investigations of the pre- and post-settlement early life of *Notolabrus* is needed to refine models of the interrelationship of environmental variables and the biology of these species, and how they manifest in their population dynamics. This study constitutes a significant advance in this direction, indicating that variability in temperature and productivity is a major factor in the development of the patterns of settlement of *Notolabrus*, and the life-history strategies it has evolved.

5. Growth of a temperate, reef-associated wrasse (*Notolabrus fucicola*), estimated from age-at-length and tag-recapture models.

Abstract. Growth of a temperate reef-associated fish, the purple wrasse, *Notolabrus fucicola*, was examined on the east coast of Tasmania using age- and length-based models. Otolith age estimates were used to construct models based on the von Bertalanffy growth function (VBGF) in the standard and a reparameterised (equivalent mathematically, but in a more biologically interpretable) form. Growth trajectories from tag-recaptures were used to construct length-based growth models, derived from the GROTAG reparameterisation of Fabens' model. Likelihood ratio tests (LRTs) were used to optimally parameterise the GROTAG models, including estimates of individual growth variability, seasonal growth, measurement error and outliers for each dataset. Growth models and parameter estimates were compared based on stratified bootstrap confidence intervals, LRTs, and 2-dimensional plots of bootstrap parameter estimates. The relative merit of these three methods for comparing models and parameters are evaluated, with LRTs combined with bootstrapping providing the most insight. Significant differences in growth of purple wrasse were found between sites in both length- and age-based models, and a large difference in growth rate between sexes was found at one site in length-based models. Seasonal and individual variability in growth was estimated successfully in the length-based models.

Introduction

Methods for estimating growth in wild fish stocks derive largely from two data sources: (1) age-based models using data for length-at-age, with fish ages known or estimated from scales, otoliths and other hard parts (e.g. the von Bertalanffy growth function or VBGF); and (2) length-based models, using recaptures of tagged fish to describe a growth trajectory over time at liberty (e.g. Fabens' transformation of the von Bertalanffy growth function for tag recapture data), or analysis of modal progressions in length-frequency data (e.g. MULTIFAN, Fournier et al. 1990). Many of these models seek to characterise mean growth of the population in terms of the three standard von Bertalanffy parameters *viz* l_∞, the theoretical asymptotic mean length; k, the growth constant; and t_0, the theoretical time at length zero.

Despite its wide use in descriptions of fish growth, the standard VBGF is often criticised. Criticisms arise from instances where the function's parameters may represent unreasonable extrapolations beyond available data and hence lack biological relevance (e.g. Knight 1968; Roff 1980; Francis 1988a; b), estimates of l_∞ produced by standard length- and age-based versions of the model lack of mathematical equivalence (e.g. Francis 1988a, 1992), and the statistical properties of the parameters make comparisons between samples difficult (Ratkowsky 1986; Cerrato 1990; 1991).

These criticisms have led to various reparameterisations of the VBGF (see Ratkowsky 1986; Cerrato 1991 for examples). Analyses of reparameterisations for age-based VBGFs suggest that the inclusion of parameters that are expected lengths-at-age, drawn from the dataset, dramatically improve the statistical properties of the model (Cerrato 1991), and also result in parameters that have direct biological interpretation. Reparameterisations which fit this criterion include Francis' (1988a) for age-at-length data, and Francis' GROTAG, a reparameterisation of Fabens' model

for tagging data (Francis 1988b). GROTAG in particular has the advantage of being readily parameterised to include seasonal growth terms, and, through the application of a likelihood function, can include estimators of measurement error, individual growth variability, and proportion of outliers in a dataset. It has been used to produce growth estimates for both cartilaginous (Francis and Francis 1992; Francis 1997; Francis and Mulligan 1998; Simpendorfer 2000; Simpendorfer *et al.* 2000) and bony fishes (Francis 1988b; Francis 1988c; Francis *et al.* 1999), and bivalves (Cranfield *et al.* 1996). Fitting of a growth model using maximum likelihood methods also provides for straight-forward comparisons between parameter estimates, and tests of the optimal parameterisation of models using likelihood ratio tests (Kimura 1980; Francis 1988a). Computationally intensive methods such as bootstrapping provide an empirical method for approximating probability distributions of growth parameter estimates (Haddon 2001), generating confidence intervals to test for differences between parameter estimates, and visualizing relationships between parameters (Mooij *et al.* 1999). Drawing together these methods, growth models can be applied that produce biologically interpretable parameter estimates and are easily fitted to growth data, and tests for comparing growth parameters can be evaluated.

The purple wrasse, *Notolabrus fucicola* is a gonochoristic, site-attached, reef associated fish, common on moderate to fully exposed coasts in south-eastern Australia and New Zealand (Russell 1988; Edgar 1997). Both *Notolabrus fucicola* and its Australian congener, the blue-throated wrasse *N. tetricus*, are large benthic carnivores, and play a significant role in the trophic dynamics of temperate reefs in their range (Shepherd and Clarkson 2001; Denny and Schiel 2001). The development of a live fishery based on *N. fucicola* and *N. tetricus* in southeastern Australia, makes temperate wrasses increasingly important economically (Lyle and Hodgson 2001).

Previous attempts to describe the growth of *N. fucicola* (Barrett 1995b; 1999) have been compromised by small sample sizes, lack of age validation, and the use of unsuitable statistical models to compare length-at-age between populations (Patterson 2000). For example, Barrett (1999) concluded that temperate reef fishes were not resource limited, as he detected no growth differences between populations, but used an inappropriate statistical method (ANCOVA), which was unlikely to detect such differences. Quantification of growth, and components of spatial and temporal growth variability, is required to progress the understanding of the population dynamics of *N. fucicola*, and provide a scientific basis for the management of this species. Variability at the individual reef scale is of particular importance as they are site attached and therefore prone to localised fishing effects. A recently validated ageing method using sectioned otoliths (Ewing *et al.* in press), and tagging data for populations from which otoliths have been obtained, enables a more detailed examination of growth in *N. fucicola*, including quantification of individual, spatial, temporal and/or sex based growth variability. This study describes site-and sex-specific age- and length-based models for this species. We also compare methods for examining differences in parameter estimates, including confidence intervals based on bootstrap estimates, plots of bootstrap estimates and likelihood ratio tests.

Materials and Methods

Field methods

Notolabrus fucicola were trapped and tagged at two sites on the east coast of Tasmania. Trapping was conducted at 1-2 month intervals, between July 1999 and April 2001 at Lord's Bluff (42.53 °S, 147.98 °E), and between July 2000 and March 2001 at Point Bailey (42.36 °S, 148.02 °E). Standard T-bar tags were inserted between the pterygiophores in the rear portion of the dorsal fin. Each fish had total length recorded to the nearest mm and was then released. As *N. fucicola* show no external sexual characters, fish could only be sexed by the presence of extruded gametes if fish were running ripe when captured, or if captured at the termination of the study.

At the conclusion of the tag-recapture study, each site was fished down. Recaptured tagged fish were euthanased by immersion in an ice slurry. Fish captured at Lord's Bluff were measured fresh, sexed, and their sagittal otoliths were removed. All fish captured at Point Bailey were processed in a similar fashion, but were stored frozen prior to examination.

Otolith preparation and interpretation

Sagittal otoliths were mounted in a block of polyester resin, and transverse sections (250-300 μm thick) cut through the primordium with a lapidary saw. Sections were mounted on a slide and examined under a binocular microscope at ×25 magnification. Annuli were counted by the primary author and individuals allocated to a year class based on the method described in Ewing *et al.* (in press). For the calculation of fractional ages, an arbitrary birth date of 1 October was assigned, based on the

approximate peak in back-calculated spawning dates of settlement stage *N. fucicola* on the east coast of Tasmania (Chapter 4).

A random subsample of 55 otoliths, including both sites, was re-aged by the author and another experienced otolith reader (GE[1]). To determine if any significant differences existed within or between reader age estimates, the frequency distributions of ages in each set of estimates was compared with a Kolmogorov-Smirnov test. Consistency of age estimates were also compared using age bias plots (Campana *et al.* 1995) and the index of average percent error (IAPE *sensu* Beamish and Fournier 1981).

Preliminary inspection of the length data for individuals from Point Bailey, where fish were frozen and thawed before subsequent measurement and otolith extraction, revealed many relatively large negative growth increments when compared to length data collected from recaptures prior to the conclusion of field sampling. Consequently, all measurements taken based on frozen fish were deemed to be incompatible with measurements taken on fresh fish, and were removed from both the tagging and otolith data sets.

Otolith age-based growth modelling

Data consisted of age estimated from otoliths (T) and length at final recapture (L). To determine whether there were differences between the proportional frequency distributions of fish lengths for which age had been estimated, a Kolmogorov-Smirnov test was conducted between sites and between sexes within sites. Growth was modelled using the standard von Bertalanffy growth function (VBGF, Table 5.1):

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[1] Graeme P. Ewing, Senior Technical Officer. Taroona Marine Research Laboratories, Tasmanian Aquaculture and Fisheries Institute, University of Tasmania.

$$L = l_{\infty}\left[\ 1 - e^{-k(T-t_0)}\ \right] \qquad (1)$$

The VBGF for the two sites and sexes within sites were modelled separately. Unsexed fish were not included in the sex specific models.

A reparameterised version of the VBGF was also estimated, based on eq. 4 in Francis (1988a):

$$L = l_{\tau} + \frac{\left[l_{\upsilon} - l_{\tau}\right]\left[\ 1 - r^{2(T-\tau)/(\upsilon-\tau)}\ \right]}{1 - r^2} \qquad (2)$$

where

$$r = \frac{l_{\upsilon} - l_{\omega}}{l_{\omega} - l_{\tau}} \qquad (3)$$

and where l_{τ}, l_{υ} and l_{ω}, are the mean lengths at ages τ, υ and $\omega=(\tau+\upsilon)/2$, the ages being chosen arbitrarily from within the observed range of ages in the dataset (Table 5.1). Consequently, these parameters are more directly observable from the data, and have more statistically favourable properties than the standard VBGF parameters l_{∞}, k and t_0 (Francis 1988a; Cerrato 1991). The values chosen for all the otolith based models were $\tau=4$, $\omega=7$ and $\upsilon=10$ years, encompassing the range of ages represented in the datasets for both sites.

Models were fitted using least squares, using the Solver add-in for Microsoft® Excel 2000. The quality of the fits was gauged in the first instance by the lack of trends in plots of residuals against length-at-age.

To determine whether growth showed any site or sex specific differences, likelihood ratio tests (LRTs) were conducted on the VBGFs and individual parameters (Kimura 1980).

Table 5.1. Abbreviations and descriptions of parameters estimated by the main model types, used in the tables and figures below. GROTAG= Francis (1988b) growth model for tagging data. VBGF= Von Bertalanffy growth function for length-at-age data, standard and as reparameterised by Francis (1988a).

	Parameters estimated	
Model	Abbreviation	Description
VBGF	l_∞ (cm)	Theoretical mean length at age ∞
	k (yr^{-1})	Growth constant
	t_0 (yr)	Theoretical time at mean length 0
	l_τ, l_υ, l_ω (cm)	Mean length at ages τ, υ and ω years, which are representative age classes in the dataset
GROTAG	g_α, g_β ($cm.yr^{-1}$)	Mean annual growth at length α and β cm, which are representative length classes in the dataset
	v	Coefficient of growth variability
	u	Extent of seasonal growth
	w (yr)	Annual peak seasonal growth, relative to 1 January
	m (cm)	Mean measurement error
	s (cm)	Standard deviation of measurement error
	p	Proportion of outliers in dataset

To further investigate the behaviour of each model, each dataset was bootstrapped 5000 times. The bootstrapping procedure involved randomly

resampling, with replacement, from the original dataset, and then fitting the VBGF to this new data set, generating new estimates of all model parameters (Haddon 2001).

The traps used to sample *N. fucicola* are size selective, resulting in individuals <20 cm and >30 cm being under-represented (Ewing unpublished data). To retain these under-represented size ranges in equal proportion in all bootstrap datasets, resampling was stratified. All data points where individuals were <20 cm and ≥30 cm (or ≥28 cm when modelling sexes within sites, to ensure that the upper size stratum had >3 individuals), and the middle size stratum, were resampled separately (Table 5.2).

Based on the percentile distribution of bootstrap parameter estimates, 95% confidence intervals (CIs) around the original sample estimates were calculated for each VBGF parameter. To account for any asymmetry in the distribution of bootstrap parameter estimates around the original estimate, a first order correction for bias of CIs was performed (Haddon 2001, pp. 171-2). The bootstrap parameter estimates were plotted against each other and compared, between sites, and between sexes within sites, to investigate correlations between model parameters,

Length-based growth modelling

Growth trajectories consisted of the initial length (L_1), time at first capture (T_1), time at final recapture (T_2), change in length from the first to the last recapture (ΔL), and duration in years between capture and last recapture (ΔT). T_1 and T_2 were measured as years from an arbitrarily chosen point, 1st January, 1999, being the first day in the earliest year in which tagging was conducted. For individuals recaptured more than once, only information relating to the initial and final captures were used in the analyses. This approach maximised the time between recaptures for any fish,

increasing the chance of detecting growth, and gives equal weight to each fish sampled.

Table 5.2. Main model types, datasets, and bootstrapped strata sizes used to produce estimates of growth for *Notolabrus fucicola*. LB= Lord's Bluff, full dataset; LB2= Lord's Bluff, only fish captured over dates equivalent to the PB data set; PB= Point Bailey, full dataset; ♂♂= males only; ♀♀= females only; All= all individuals, including unsexed fish. *N*= sample size.

			Bootstrap strata *N*		
Model	Dataset	Total *N*	Lower	Middle	Upper
GROTAG	LB2 All	174	27	137	10
	PB All	263	58	183	22
	LB ♂♂	103	23	66	14
	LB ♀♀	70	11	47	12
	PB ♂♂	97	23	59	15
	PB ♀♀	88	15	60	13
VBGF	LB All	101	9	82	10
	PB All	178	32	132	14
	LB ♂♂	47	6	33	8
	LB ♀♀	54	4	38	12
	PB ♂♂	68	8	49	11
	PB ♀♀	104	17	69	18

The two sites where *N. fucicola* were tagged were sampled over different time periods. For the purposes of between site growth comparisons, only data from Lord's Bluff contemporary with data from Point Bailey were considered (Table 5.2). Thus this dataset, designated LB2, reduced potentially confounding effects of longer sampling durations at Lord's Bluff.

A Kolmogorov-Smirnov test was conducted between sites and between sexes within sites, to determine whether differences existed between the proportional frequency distributions of lengths of fish at first capture (L_1).

Growth was modelled using GROTAG (based on eqs. 2 and 4 respectively, in Francis (1988b)), a reparameterisation and extension of the Fabens' growth model for tag-recapture data, in a form incorporating seasonal growth (Table 5.1):

$$\Delta L = \left[\frac{\beta g_\alpha - \alpha g_\beta}{g_\alpha - g_\beta} - L_1 \right] \left[1 - \left(1 + \frac{g_\alpha - g_\beta}{\alpha - \beta} \right)^{\Delta T + (\phi_2 - \phi_1)} \right] \qquad \textbf{(4)}$$

where

$$\phi_i = u \frac{\sin[2\pi(T_i - w)]}{2\pi} \qquad \textbf{(5)}$$

for i= 1,2.

The parameters g_α and g_β are the estimated mean annual growth (cm.yr^{-1}) of fish of initial lengths α cm and β cm respectively, where $\alpha<\beta$. The lengths α and β were chosen such that the majority of values of L_1 in each dataset fell between them (Francis 1988b). For site specific estimates of growth, α and β were set at 20 cm and 30 cm respectively, while for sexes within sites, α and β were 20 cm and 28 cm. Seasonal growth is parameterised as w (the fraction of the year from 1 January when growth is at its maximum) and u (estimated so that the maximum and minimum growth rates resulting from any season growth variability are related in the ratio (1 + u) : (1 - u).

The model was fitted by maximising of a likelihood (λ) function (based on Francis' eq. 9 (1988b)) using the Solver add-in for Microsoft® Excel 2000. For each dataset, made up of $i = 1$ to n growth increments:

$$\lambda = \Sigma_i \ln\left[(1-p)\lambda_i + p/R\right] \tag{6}$$

where

$$\lambda_i = \exp\frac{-\frac{1}{2}\left(\Delta L_i - \mu_i - m\right)^2 / \left(\sigma_i^2 + s^2\right)}{\left[2\pi\left(\sigma_i^2 + s^2\right)\right]^{\frac{1}{2}}} \tag{7}$$

The measured growth increment of the i-th fish, ΔL_i, has its corresponding expected mean growth increment, μ_i, as determined from equation 4 above, where μ_i is normally distributed with standard deviation σ_i. In this study, growth variability, v, was parameterised to make σ_i a function of the expected growth increment μ_i (eq. 5, Francis 1988b):

$$\sigma_i = v\mu_i \tag{8}$$

The estimate of individual growth variability (v) is estimated as a scaling factor of the standard deviation, assuming a monotonic increase in individual variability around the mean growth increment, as the size of the increment increases.

In its fully parameterised form, the likelihood function estimates the population measurement error in ΔL as being normally distributed, with a mean of m and standard deviation of s. To estimate the proportion of outliers, Francis (1988b) also includes p, the probability that the growth increment for any individual could exist erroneously in the dataset as any value, within the observed range of growth

increments R. This enables the number of outliers to be identified and their potential influence of on growth parameter estimates to be taken into account.

The optimal model parameterisation was determined by fitting 5 different models, comprising different combinations of parameters (Table 5.3). A likelihood ratio test (LRT) was used to determine the improvement in model fit with the different parameterisations. If the net decrease in the negative log likelihood ($-\lambda$) of a model was beyond a critical value (i.e. 1.92 for one extra fitted parameter, 3.00 for a two parameter increase), relative to the $-\lambda$ of a model with less parameters, it represented a significant improvement in fit at the 5% level (Francis 1988b). For models with an equal number of parameters, the model producing the lowest $-\lambda$ was judged the best model.

Table 5.3. Parameters estimated in the 5 GROTAG models fitted to each tag-recapture dataset.

GROTAG Model	Parameters estimated
1	g_α, g_β, v, p
2	$g_\alpha, g_\beta, v, p, u, w$
3	$g_\alpha, g_\beta, v, p, s, m$
4	$g_\alpha, g_\beta, v, p, u, w, s, m$
5	$g_\alpha, g_\beta, v, u, w, s, m$

As with the otolith model comparisons, LRTs were conducted on the GROTAG models between sites and sexes within sites. Models were also bootstrapped 5000 times, with stratification as described above for otolith based models (Table 5.2). First order corrected 95% CIs were calculated for parameter

estimates (Haddon 2001), and plots of values and correlations of bootstrap parameter estimates were compared between sites and sexes within sites.

Results

Otolith interpretation

A Kolmogorov-Smirnov test showed no significant differences in age-frequency distributions generated by repeat reads of 55 otoliths by the primary author ($D_{0.05}$ =0.259, D_{max}=0.072, ns). Similarly, no significant differences were found between the age-frequency distribution resulting from the author's primary reads, and another experienced reader interpreting the same 55 samples ($D_{0.05}$ =0.259, D_{max}=0.109, ns). The IAPE score for all 3 readings was calculated as 6.9%, and no strong biases were apparent in age bias plots within or between readers. So, age estimates derived from the first readings by the author were used for modelling.

Age-based growth modelling

Site comparisons

No significant differences in length frequencies were detected in a Kolmogorov-Smirnov test between sites in the length-at-age data (PB All vs LB All, D_{crit} =0.169, D_{max}=0.097, ns), so any differences in growth models could be unambiguously interpreted as differences in length-at-age.

Notolabrus fucicola length-at-age estimates showed high variability among individuals (Figure 5.1). Mean lengths-at-age were adequately described by the von Bertalanffy growth function (VBGF), across the ages represented by the samples from

the two sites. The plots of the site-specific VBGFs suggested that mean length-at-age at Lord's Bluff was higher than at Point Bailey (Figure 5.1).

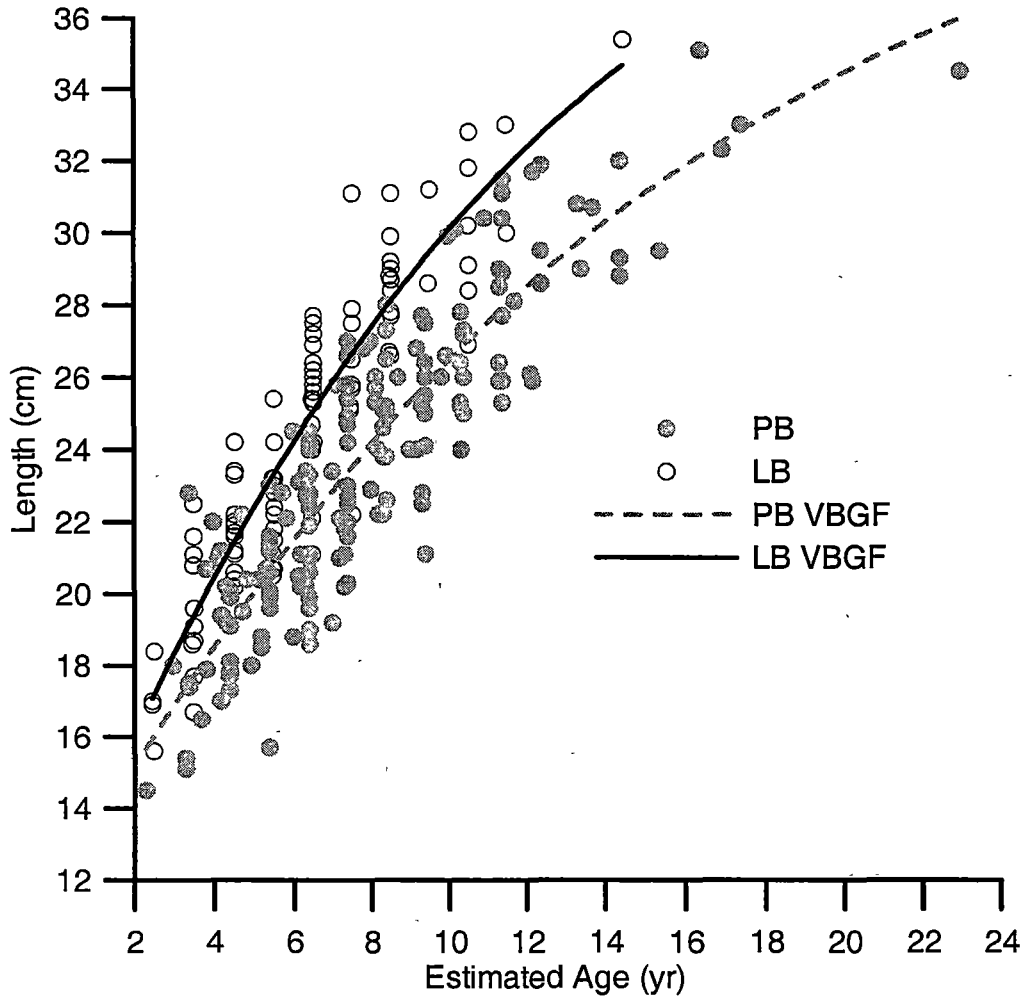

Figure 5.1. Age-at-length estimates for *Notolabrus fucicola*, derived from otoliths (symbols), and corresponding von Bertalanffy growth functions (VBGFs) fitted by least squares (lines). PB = Point Bailey south, LB = Lord's Bluff.

Due to the absence of young (0+ and 1+) fish in the samples from both sites, and old fish at Lord's Bluff, the standard VBGF estimate of t_0 and l_∞ have no biological interpretation (Table 5.4). Confidence intervals for the three standard VBGF parameters overlapped in comparisons between sites (Table 5.4). Scatter plots

of the bootstrap parameter estimates show strong non-linear correlations between parameters, particularly in the relationship between estimates of l_∞ and k (Figure 5.2). Bootstrap estimates of l_∞ and k revealed minimal overlap between sites, most easily visualised with logarithmic axes (Figure 5.2 a). Likelihood ratio tests (LRTs) showed that differences between sites were highly significant overall, but could not be attributed to significant differences in individual parameters (Table 5.5).

Table 5.4. Von Bertalanffy growth function parameter estimates for *Notolabrus fucicola*. Bold text are parameter estimates from the original dataset. Text in parentheses are the proportion of parameter estimates from bootstrapped datasets that were less than the estimate from the original data set. Plain text are first-order corrected bootstrap 95% confidence intervals.

	Parameter estimate					
Dataset	l_∞ (cm)	k (yr^{-1})	t_0 (yr)	l_4 (cm)	l_7 (cm)	l_{10} (cm)
LB All	**44.7**	**0.085**	**-3.23**	**20.4**	**25.9**	**30.1**
	(0.49)	(0.51)	(0.49)	(0.50)	(0.48)	(0.49)
	36.0 to 69.5	0.037 to 0.149	-5.68 to -1.61	20.0 to 20.9	25.5 to 26.2	29.4 to 30.7
PB All	**43.3**	**0.065**	**-4.65**	**18.5**	**22.9**	**26.5**
	(0.66)	(0.38)	(0.45)	(0.48)	(0.55)	(0.72)
	37.6 to 207.9	0.013 to 0.096	-8.93 to -2.75	17.9 to 19.0	22.6 to 23.2	26.2 to 26.9
LB♂♂	**52.1**	**0.059**	**-4.46**	**20.3**	**25.5**	**29.7**
	(0.53)	(0.48)	(0.47)	(0.52)	(0.47)	(0.50)
	34.5 to 1060.0	0.001 to 0.156	-8.81 to -1.52	19.8 to 20.9	24.9 to 26.0	28.9 to 30.5
LB♀♀	**43.2**	**0.095**	**-2.80**	**20.5**	**26.1**	**30.4**
	(0.38)	(0.60)	(0.58)	(0.60)	(0.58)	(0.53)
	32.7 to 68.9	0.035 to 0.209	-5.72 to -0.71	20.1 to 21.3	25.5 to 26.8	29.4 to 31.7
PB♂♂	**43.3**	**0.060**	**-5.56**	**18.5**	**22.9**	**26.5**
	(0.48)	(0.51)	(0.50)	(0.53)	(0.51)	(0.50)
	33.3 to 126.6	0.010 to 0.125	-11.10 to -2.50	18.3 to 19.4	22.5 to 23.4	25.7 to 26.8
PB♀♀	**43.2**	**0.065**	**-4.60**	**18.9**	**22.9**	**26.3**
	(0.47)	(0.53)	(0.52)	(0.50)	(0.54)	(0.50)
	37.0 to 159.0	0.009 to 0.104	-10.02 to -2.34	17.7 to 19.3	22.6 to 23.3	26.1 to 27.0

Confidence intervals for the Francis (1988a) reparameterised version of the VBGF clearly indicated differences in growth rates between sites in all three parameters, with no overlap between sites in the estimates of mean length at 4, 7 or 10

years old (Table 5.4). These differences were also evident in plots of bootstrap parameter estimates, the two sites being clearly separated in the parameter space (Figure 5.3). These parameters showed none of the highly non-linear correlation evident in the standard VGBF estimates (Figure 5.3 b). Highly significant differences in all individual parameters were evident in LRTs between PB All and LB All (Table 5.5).

Table 5.5. Likelihood ratio tests of site differences in the von Bertalanffy growth functions fitted to *Notolabrus fucicola* age-at-length data and individual VBGF parameters, both standard and reparameterised. RSS= residual sum of squares. The base case represents the RSS for both curves fitted separately.

Hypothesis	RSS	χ^2	df	*P*
Base case	868	-	-	-
Coincident curves	1369	127.25	3	<0.001
$= l_\infty$	868	0.03	1	0.870
$= k$	869	0.32	1	0.573
$= t_0$	877	2.99	1	0.084
$= l_4$	948	1.07	1	0.001
$= l_7$	1240	43.22	1	<0.001
$= l_{10}$	1125	31.39	1	<0.001

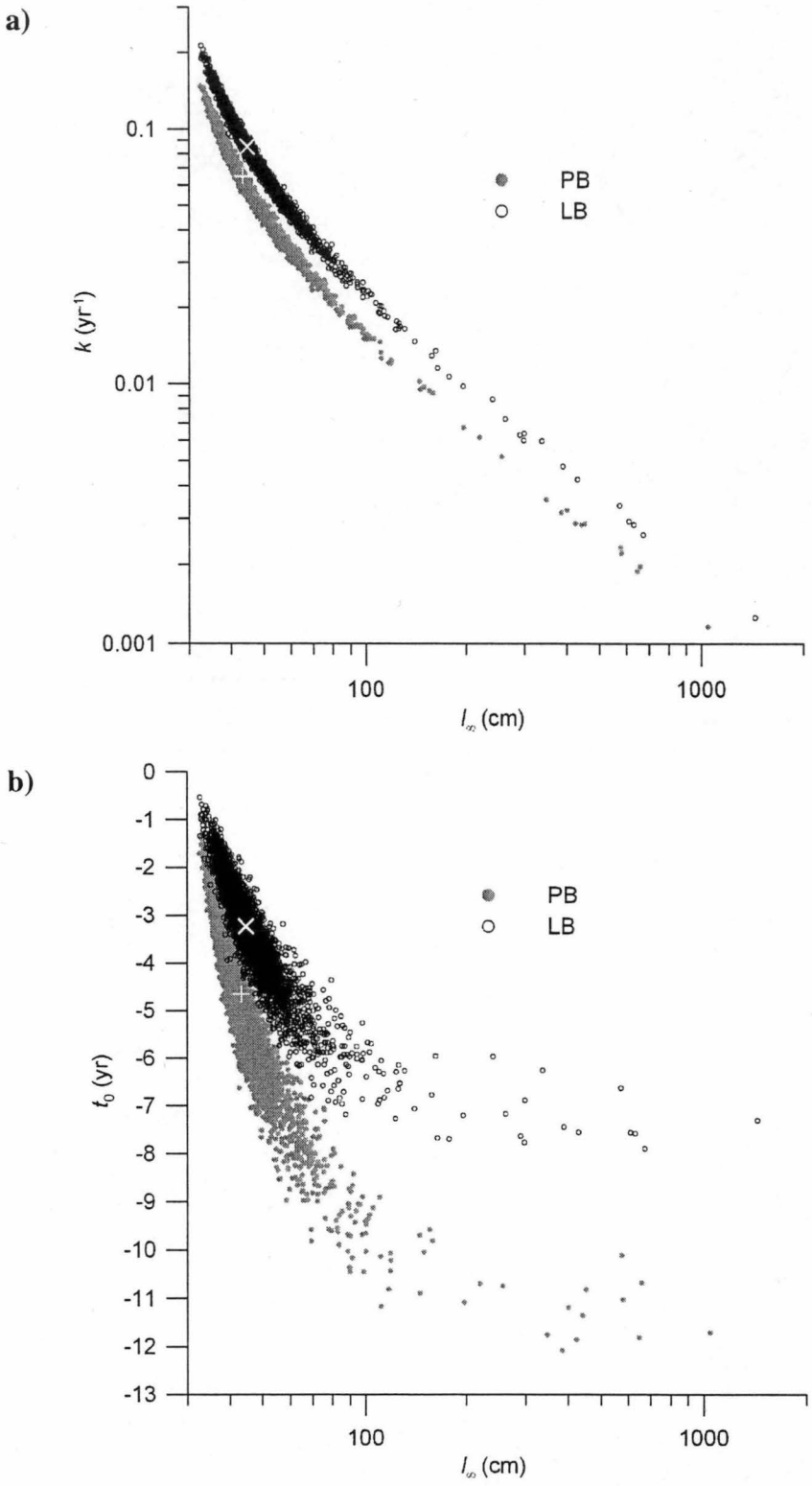

Figure 5.2. Bootstrap parameter estimates for *Notolabrus fucicola*, by site, for the standard von Bertalanffy growth function. Note l_∞ and k are plotted on logarithmic axes for clarity : **a)** l_∞ vs k **b)** l_∞ vs t_0 **c)** k vs t_0. Contrasting crosses show the location of parameter estimates based on the original data set (+, PB = Point Bailey south, ×, LB= Lord's Bluff).

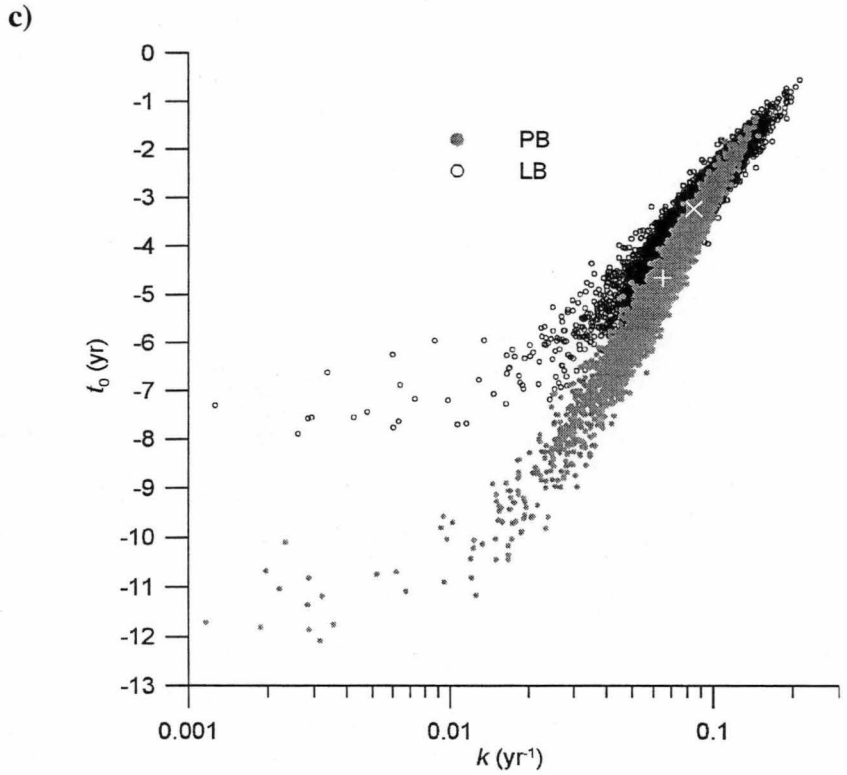

Figure 5.2. cont.

Sex comparisons

Confidence intervals for the standard and reparameterised von Bertalanffy parameters overlapped in all comparisons between sexes within sites (Table 5.4). Likelihood ratio tests showed no significant differences between models of sexes within sites, a conclusion supported by considerable overlap in plots of bootstrap estimates.

Length-based growth modelling

Model parameterisation

Site-specific datasets were optimally parameterised under the most complex model (model 4), incorporating seasonal growth and measurement error estimates (Table 5.6). Estimates of proportion of outliers in the dataset (p) greater than zero were due to lack of fit, and dropped to zero in the most complex model. Model 5 (equivalent to

model 4 with p held at zero), was the model subsequently fitted to all bootstrap datasets of LB2 All and PB All.

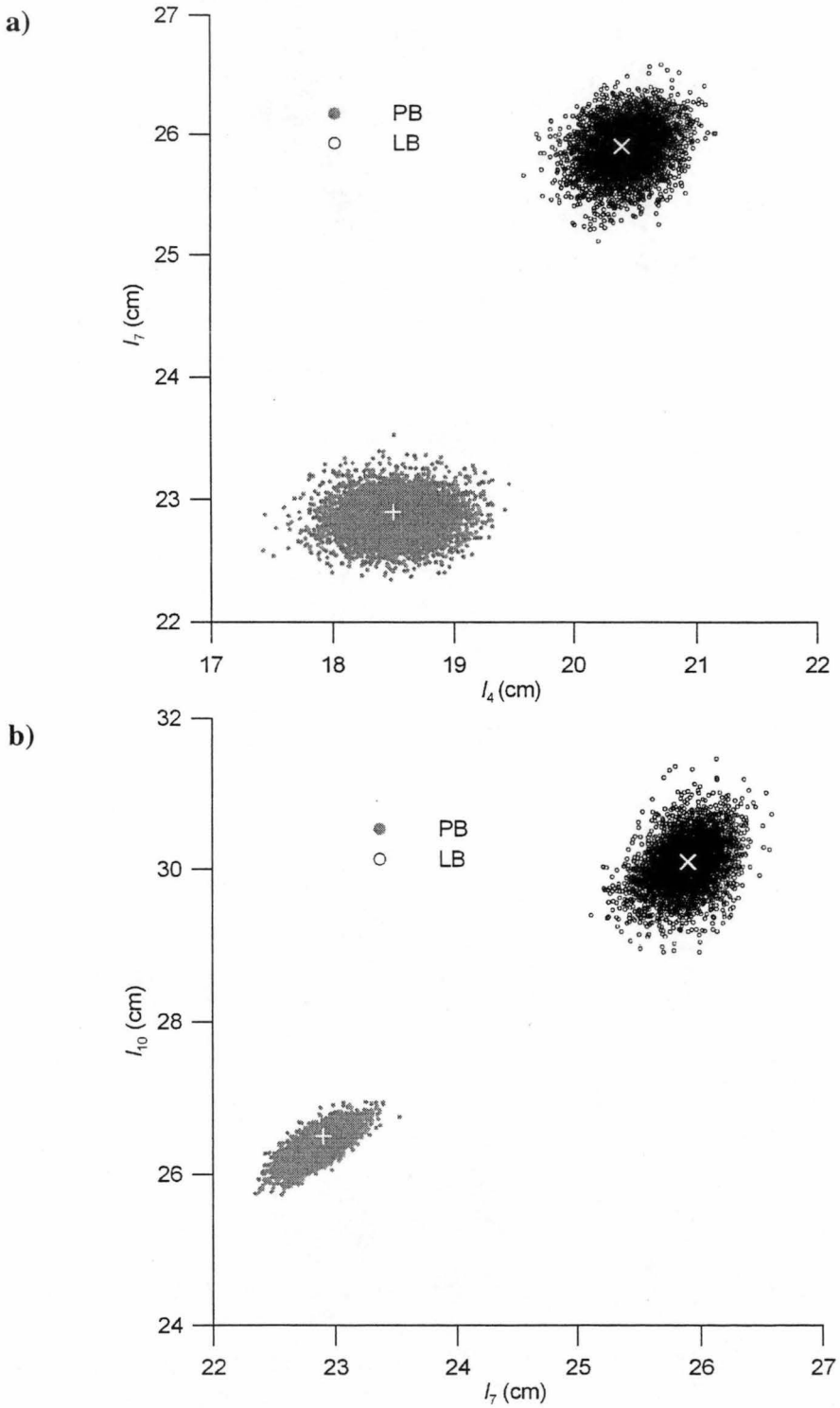

Figure 5.3. Bootstrap estimates of reparameterised von Bertalanffy growth function mean lengths at age for *Notolabrus fucicola*, by site. **a)** l_4 vs l_7 **b)** l_7 vs l_{10}. Contrasting crosses show the location of parameter estimates based on the original data set (+, PB = Point Bailey south, ×, LB= Lord's Bluff).

Table 5.6. Parameter estimates and negative log-likelihoods ($-\lambda$) of models used in likelihood ratio tests to determine the optimal parameterisation of GROTAG models for *Notolabrus fucicola* tagging data, by site. Parameters are as described in Table 5.1.Bold text in $-\lambda$ column indicate the optimally parameterised model for each dataset.

Dataset	Model	Parameter estimate								
		g_{20} (cm.yr^{-1})	g_{30} (cm.yr^{-1})	v	u	w (yr)	s (cm)	m (cm)	p	$-\lambda$
LB2 All	1	1.84	1.07	0.88	-	-	-	-	0.07	57.06
	2	3.00	1.67	0.88	0.59	0.22	-	-	0.07	50.46
	3	2.60	1.12	0.29	-	-	0.22	-0.12	0.00	20.59
	4	3.30	1.42	0.26	0.45	0.14	0.22	-0.10	0.00	**12.97**
PB All	1	1.50	1.01	0.73	-	-	-	-	0.16	87.82
	2	1.55	1.15	0.82	0.31	0.13	-	-	0.07	79.02
	3	1.87	1.18	0.36	-	-	0.19	-0.08	0.00	36.16
	4	1.53	1.01	0.35	0.57	0.91	0.18	-0.07	0.00	**23.52**

Model 4 provided the optimal parameterisation for sex-specific datasets, (Table 5.7). With the exception of LB♀♀ where $p = 0.03$, estimates of p dropped to zero in model 4. Holding p=0 (equivalent to model 5) for LB♀♀ resulted in a reduction in the negative log-likelihood ($-\lambda$) of the model relative to model 4. Model 5 changed parameter estimates slightly, increasing growth (g_{20} and g_{28}), growth variability (v), and measurement error (m) relative to model 4 (Table 5.7). However, model 5 still constituted a significant improvement in fit over models 1-3, and improved the stability of the model fitting procedure under bootstrapping. So model 5 was chosen to be fitted to all bootstrap datasets for comparisons between the sexes at Lord's Bluff, and at Point Bailey.

Table 5.7. Parameter estimates and negative log-likelihoods ($-\lambda$) of models used in likelihood ratio tests to determine the optimal parameterisation of GROTAG models for *Notolabrus fucicola* tagging data, by sex within site. Parameters are as described in Table 5.1. Bold text in $-\lambda$ column indicate the optimally parameterised model for each dataset, † indicates the $-\lambda$ value for a near-optimal model, subsequently bootstrapped.

		Parameter estimate								
Dataset	Model	g_{20} (cm.yr^{-1})	g_{28} (cm.yr^{-1})	v	u	w (yr)	s (cm)	m (cm)	p	$-\lambda$
LB♀♀	1	2.05	1.40	0.52	-	-	-	-	0.16	60.58
	2	1.99	1.20	0.48	0.41	0.98	-	-	0.15	58.19
	3	2.88	1.87	0.26	-	-	0.25	-0.29	0.00	41.15
	4	2.66	1.48	0.22	0.47	0.94	0.22	-0.26	0.03	**36.23**
	5	2.75	1.75	0.25	0.32	0.96	0.24	-0.31	-	38.40†
LB♂♂	1	1.98	1.49	0.52	-	-	-	-	0.00	43.07
	2	1.88	1.54	0.50	0.23	0.04	-	-	0.00	39.24
	3	2.09	1.62	0.27	-	-	0.21	-0.05	0.00	32.21
	4	2.04	1.67	0.27	0.23	0.19	0.20	-0.04	0.00	**29.44**
PB♂♂	1	1.31	1.02	0.60	-	-	-	-	0.24	21.31
	2	1.15	0.96	0.61	0.41	0.90	-	-	0.19	19.93
	3	1.54	1.21	0.33	-	-	0.19	-0.03	0.00	6.43
	4	1.15	0.93	0.32	0.81	0.88	0.18	-0.04	0.00	**2.49**
PB♀♀	1	1.49	1.15	0.68	-	-	-	-	0.16	30.55
	2	1.43	1.16	0.90	0.33	0.12	-	-	0.00	28.85
	3	1.96	1.32	0.38	-	-	0.20	-0.11	0.00	19.06
	4	1.46	1.01	0.39	0.77	0.87	0.18	-0.12	0.00	**15.78**

Site comparisons

With the exception of s in the LB2 All dataset, the proportion of bootstrap parameter estimates were evenly distributed around the original parameter estimates, resulting in approximately symmetrical first order corrected 95% CIs (Table 5.8). Based on the lack of overlap of CIs, only g_{20} differed significantly between sites.

Site differences in growth were also indicated in the results of LRTs. The overall models were significantly different, with the growth parameter g_{20} and the timing of maximum seasonal growth (w) significantly different when tested individually (Table 5.9a).

Plots of bootstrap parameter estimates clearly indicate growth rate differences between sites, with little overlap in the parameter clouds along the g_{20} axis (Figure 5.4 a). Plotting mean annual growth against initial length, based on the complete datasets, shows the faster initial growth at lord's Bluff, with decreasing difference in mean annual growth between larger fish at the two sites (Figure 5.5).Plots of the seasonal growth parameters u and w show a high level of non-linear correlation. Figure 5.4 b shows a large region of overlap between site estimates along the w axis, contradicting the single parameter LRT prediction that w is different between sites (Table 5.9 a). Estimates of the timing of seasonal growth (w) ranged from 0.85 to 1.21 for the two sites, corresponding to peak growth in *N. fucicola* between mid-spring and late summer in the southern hemisphere.

Sex comparisons

Bootstrap parameter estimates from sex-specific datasets were approximately symmetrical about the original estimates (Table 5.8). The largest divergence from 0.5 was evident in estimates of s (the standard deviation of measurement error) for LB♀♀

and PB♂♂, and u (the extent of seasonal growth variability) for LB♀♀. Estimates of u for LB♀♀ extended into unrealistic negative values at the extremes of its distribution when bootstrapped (Table 5.8).

Table 5.8. GROTAG parameter estimates derived from *Notolabrus fucicola* tag-recapture data. Parameters are as described in Table 5.1. For all datasets, g_α is the mean annual growth of individuals with an initial length of 20 cm. g_β represents the estimated mean annual growth of individuals with an initial length of 30 cm for LB2 All and PB All, or the estimate for 28 cm individuals for all other datasets. Bold text are the parameter estimates from the original datasets. Text in parentheses are the proportion of parameter estimates from bootstrap datasets less than the original estimate. Plain text are first-order corrected bootstrap 95% confidence intervals.

	Parameters estimate						
Dataset	g_α (cm.yr^{-1})	g_β (cm.yr^{-1})	v	u	w (yr)	s (cm)	m (cm)
LB2 All	**3.30**	**1.42**	**0.26**	**0.45**	**1.14**	**0.22**	**-0.10**
	(0.50)	(0.50)	(0.52)	(0.43)	(0.49)	(0.60)	(0.47)
	2.24 to 4.34	0.83 to 2.19	0.13 to 0.39	0.23 to 0.68	0.95 to 1.21	0.19 to 0.26	-0.17 to -0.03
PB All	**1.53**	**1.01**	**0.35**	**0.57**	**0.91**	**0.18**	**-0.07**
	(0.51)	(0.53)	(0.55)	(0.48)	(0.53)	(0.56)	(0.50)
	1.12 to 1.94	0.72 to 1.31	0.27 to 0.45	0.26 to 1.23	0.85 to 1.04	0.15 to 0.22	-0.12 to -0.02
LB♂♂	**2.04**	**1.68**	**0.27**	**0.23**	**1.19**	**0.20**	**-0.04**
	(0.49)	(0.51)	(0.58)	(0.46)	(0.48)	(0.55)	(0.49)
	1.77 to 2.31	1.33 to 2.00	0.20 to 0.40	0.06 to 0.43	0.95 to 1.29	0.12 to 0.27	-0.14 to 0.05
LB♀♀	**2.75**	**1.75**	**0.25**	**0.32**	**0.96**	**0.24**	**-0.31**
	(0.51)	(0.53)	(0.58)	(0.34)	(0.53)	(0.67)	(0.46)
	2.34 to 3.20	1.28 to 2.29	0.17 to 0.36	-0.10 to 0.61	0.81 to 1.22	0.15 to 0.40	-0.47 to -0.14
PB♂♂	**1.15**	**0.93**	**0.32**	**0.81**	**0.88**	**0.18**	**-0.04**
	(0.49)	(0.50)	(0.54)	(0.51)	(0.50)	(0.63)	(0.49)
	0.43 to1.82	0.37 to 1.52	0.16 to 0.47	0.18 to 4.37	0.81 to 1.15	0.14 to 0.24	-0.14 to -0.06
PB♀♀	**1.46**	**1.01**	**0.39**	**0.77**	**0.87**	**0.18**	**-0.12**
	(0.54)	(0.54)	(0.56)	(0.47)	(0.54)	(0.57)	(0.47)
	0.61 to 2.39	0.44 to 1.67	0.21 to 0.70	0.14 to 3.94	0.81 to 1.19	0.09 to 0.28	-0.23 to -0.02

Table 5.9. Likelihood ratio tests of the GROTAG models for which bootstrap parameter estimates were generated (Tables 5.6, 5.7): **a)** PB against LB2 **b)** LB♀♀ against LB♂♂. $-\lambda$= negative log-likelihoods. The base case is the negative log-likelihood of the datasets fitted with two wholly separate models. Parameters are as described in table 5.1

a)

Hypothesis	$-\lambda$	χ^2	df	P
Base case	36.49	-	-	-
Coincident curves	51.98	30.98	8	<0.001
$=g_{20}$	42.19	11.38	1	<0.001
$=g_{30}$	37.36	1.72	1	0.189
$=v$	37.35	1.72	1	0.190
$=u$	36.62	0.12	1	0.623
$=w$	38.91	4.84	1	0.028
$=s$	37.64	2.28	1	0.130
$=m$	36.66	0.33	1	0.565

b)

Hypothesis	$-\lambda$	χ^2	df	P
Base Case	67.84	-	-	-
Coincident Curves	77.40	19.11	8	0.014
$=g_{20}$	72.12	8.56	1	0.003
$=g_{30}$	67.89	0.09	1	0.768
$=v$	67.89	0.09	1	0.768
$=u$	67.96	0.23	1	0.633
$=w$	69.22	2.77	1	0.096
$=s$	68.05	0.41	1	0.523
$=m$	71.63	7.58	1	0.006

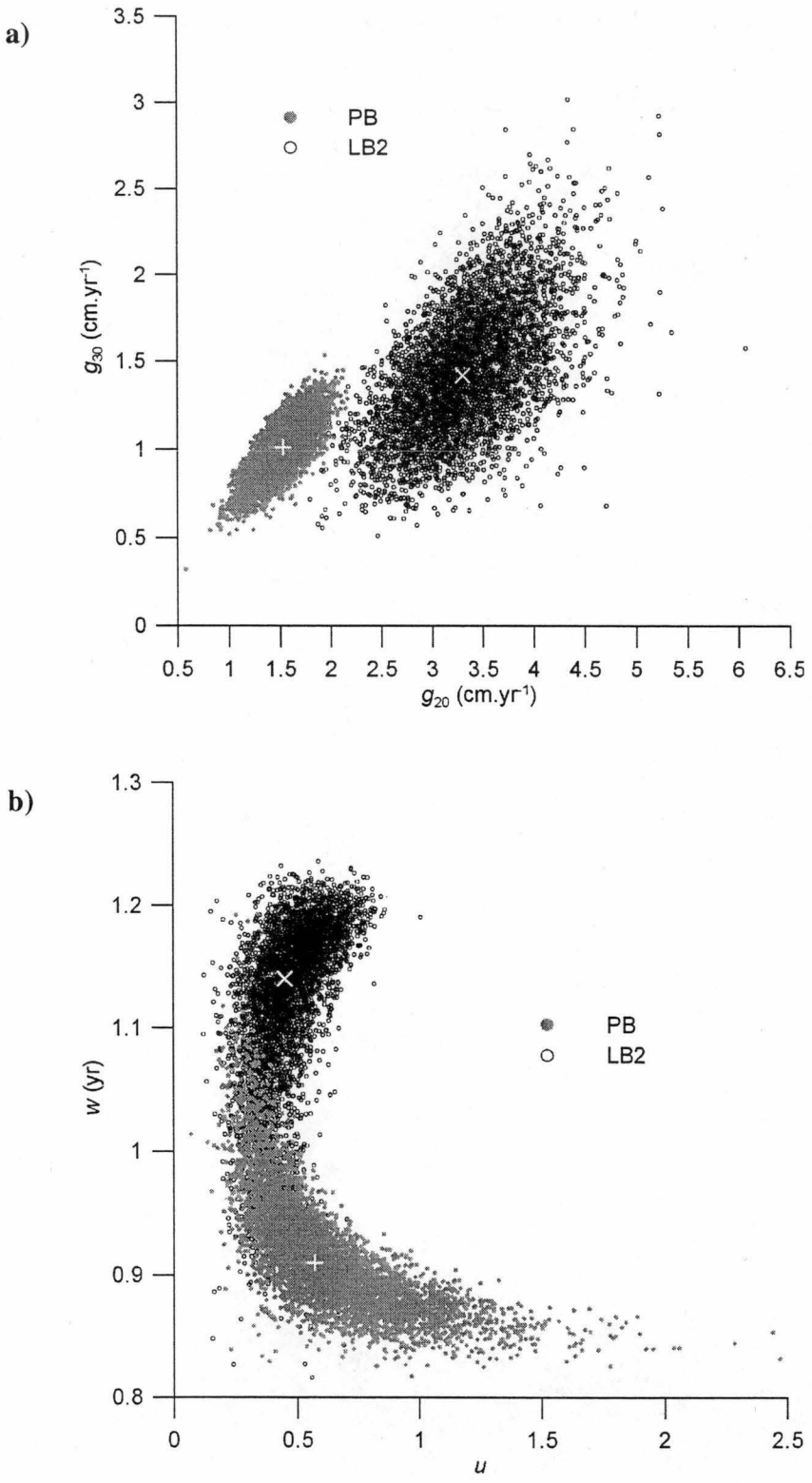

Figure 5.4. Bootstrap estimates of GROTAG parameters for *Notolabrus fucicola*, by site: **a)** g_{20} vs g_{30}, and **b)** u vs w. Parameters are as described in Table 5.1. Contrasting crosses show the location of parameter estimates based on the original data set (+, PB = Point Bailey south, ×, LB2= Lord's Bluff).

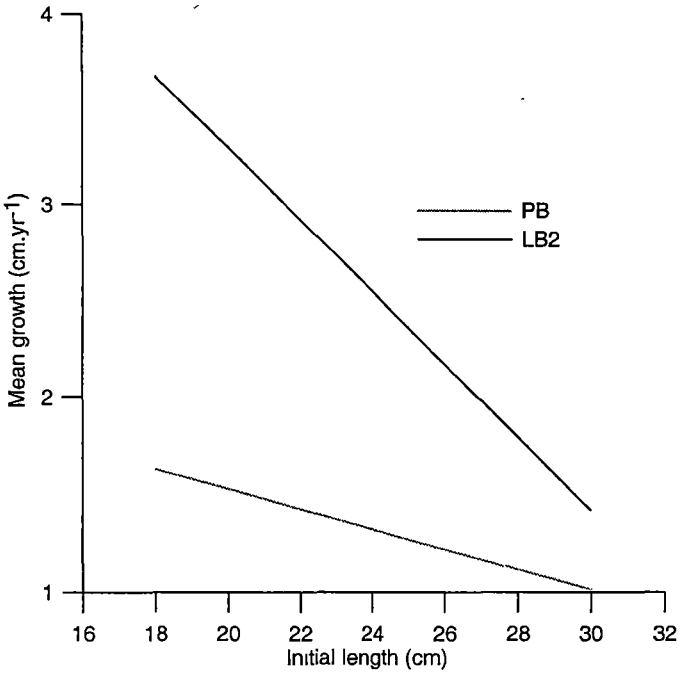

Figure 5.5. Initial lengths plotted against mean annual growth, estimated from tag-recapture data, for *Notolabrus fucicola* at Lord's Bluff (LB2) and Point Bailey (PB).

Based on confidence intervals, g_{20} and m differed significantly between LB♀♀ and LB♂♂ (Table 5.8). This conclusion is supported by LRTs, also indicating significant differences between g_{20} and m (Table 5.9b). This contrasts with the results of age-based modelling of sex-specific growth at Lord's Bluff, where no differences between the sexes was detected. Plots of the bootstrap estimates of the growth parameters g_{20} and g_{28} show clear separation between LB♂♂ and LB♀♀ along the g_{20} axis, but not along the g_{28} axis(Figure 5.6 a), corroborating conclusions drawn from the LRT and CI comparisons. Plotting mean annual growth against initial length, based on the complete dataset, shows the faster initial growth of females at lord's Bluff, with decreasing difference in mean annual growth between larger males and females (Figure 5.7). Plots of bootstrapped estimates of the measurement error

parameters s and m show separation between the two sexes in the m axis (Figure 5.6 b), also concurring with the difference in m detected by the LRT and the lack of overlap in CIs between sexes in this parameter.

Sex comparisons at Point Bailey detected no sex specific growth differences, with CIs, LRTs and bootstrap plots indicating no significant difference in any of the model parameters.

Discussion

Model comparisons

In this study, two methods, based on mathematically different concepts, produced qualitatively similar conclusions, namely that growth in the age and length classes available in this study was faster at Lords' Bluff than at Point Bailey. The results of length-based and age-based models also produced similar conclusions regarding methods suitable for robust comparisons of growth models and parameter estimates for different groups of fish (Table 5.10). Confidence intervals were only reliable indicators of difference in cases where parameters showed low levels of correlation, such as in the reparameterised von Bertalanffy growth function. Likelihood ratio tests in general provided a robust method of testing differences between models and individual parameters but, again, only where model parameters are not highly correlated. Thus, when parameters are highly correlated, as with the standard VBGF parameters l_∞ and k (Figure 5.2) and the GROTAG seasonal growth parameters u and w (Figure 5.4b), the LRT was oversensitive, predicting significant differences between parameter estimates from different groups. Bootstrapping

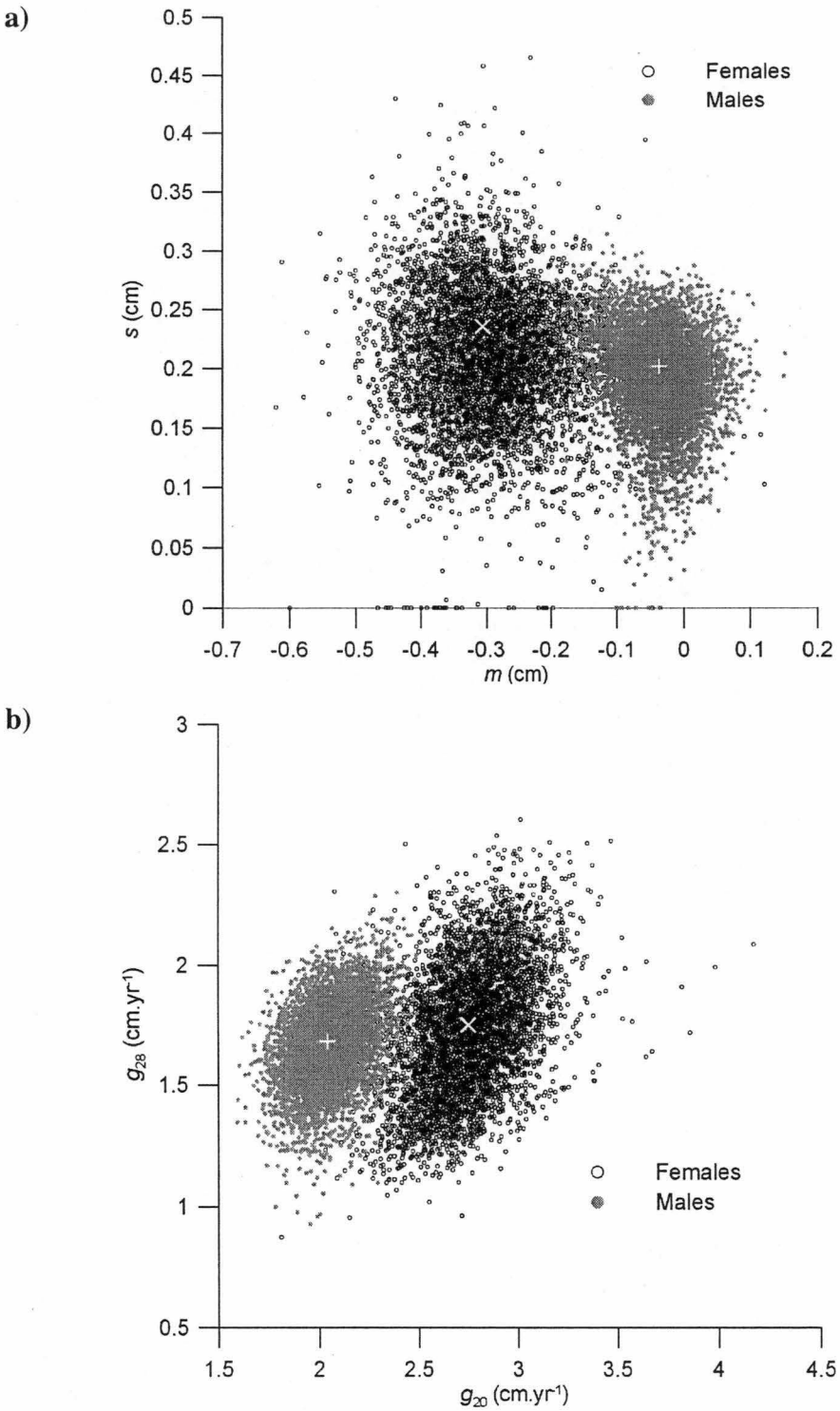

Figure 5.6. GROTAG bootstrap parameter estimates for *Notolabrus fucicola* from Lord's Bluff, by sex: **a)** g_{20} vs g_{28}, mean annual growth at initial length 20 and 28 cm; **b)** m vs s, mean and standard deviation of measurement error. Contrasting crosses show the location of parameter estimates based on the original data set (+ = Males, × = Females).

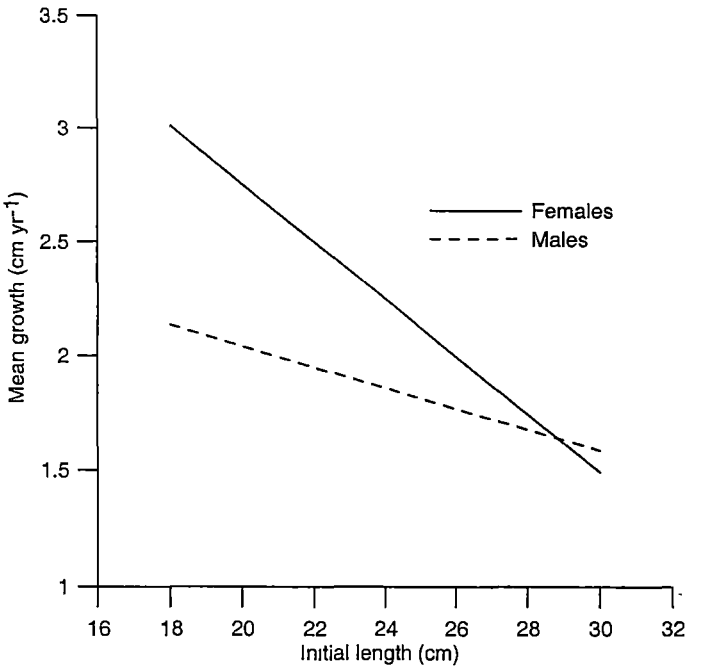

Figure 5.7. Initial lengths plotted against mean annual growth, estimated from tag-recapture data, for male and female *Notolabrus fucicola* at Lord's Bluff.

techniques proved to be a useful way of visualising the behaviour of models and empirically examining growth parameter differences, and hence are recommended as a method to complement the 'traditional' statistical tests (Table 5.10).

The difficulty in extracting biological meaning from the parameters of the standard VBGF is a common criticism of this model (e.g. Knight 1968; Roff 1980; Francis 1988a; Francis 1992). The problem is particularly acute where only a part of the size and/or age range of animals is available, a situation regularly faced in analyses of fisheries data (Haddon 2001). This study is a case in point, where the bootstrap estimates of l_∞ and t_0 for *N. fucicola* appear unrealistic (Figure 5.2 a). Due to the selectivity of the traps used in this study, the curve defined by the VBGF can only be representative for the age classes present in the datasets, and hence any attempt to interpret or compare l_∞ or t_0 as descriptors of the growth of *N. fucicola* would be

spurious. Furthermore, since k and l_∞ are highly correlated, comparisons of k cannot be independent of the effects of size or age selectivity on a dataset.

Table 5.10. Summary of suitability of different methods to compare growth models and individual parameter estimates for different groups of fish in this study. LRT = likelihood ratio tests, CIs = overlap in confidence intervals. Estimate plots are 2D plots of parameter estimates resulting from bootstrapped datasets. ✗= unsuitable, ✓= suitable, ✗/✓= unsuitable under conditions of high parameter correlation.

	Comparison method			
	Whole model	Model parameters		
Model	LRT	LRT	CIs	Estimate plots
GROTAG	✓	✗/✓	✓	✓
VBGF	✓	✗	✗	✓
Reparameterised VBGF	✓	✓	✓	✓

Variability in growth

Models of growth can lead to estimation of length dependant processes in fish populations, such as reproductive output, increases in biomass due to individual growth, selectivity of fishing gear, and the impact and appropriateness of size limits as management tools. The results of this study quantify mean growth in *N. fucicola* (e.g. Figures 5.1, 5.5 and 5.7), but also demonstrate that growth varies across individuals, seasons, sexes, and sites.

Although the significance of including v (estimating the variability in growth around the population mean) was not explicitly tested during model parameterisation, values of around 0.2 to 0.7 were estimated for all data sets modelled. Values in this range have been estimated using GROTAG from other species of bony fishes (Francis

1988a; b; c; Francis *et al.* 1992; Francis *et al.* 1999) and cartilaginous fishes (Francis and Francis 1992; Francis 1997; Francis and Mulligan 1998; Simpendorfer 2000; Simpendorfer *et al.* 2000), indicating that considerable variability in growth, presumably made up of components of growth variability within and between individuals around the population mean, is a common phenomenon. The spread of datapoints around the fitted VBGFs for *N. fucicola* concurs with the GROTAG results in this study (Figure 5.1), indicating that considerable variability in growth is characteristic of groups of *N. fucicola*, in size-at-age and in annual growth of size classes.

Variability in growth of individuals around the population mean may obscure other sources of growth variation, perhaps in part explaining why age-based models failed to detect any significant effect of sex on growth rates in this study, where length-based modelling indicated that females grow faster than males at Lord's Bluff (Figure 5.6a). Notably, mean lengths-at-age from the reparameterised VBGF indicate that females are larger than males of the same age at Lord's Bluff though this difference was not significant (Table 5.4). It is possible, with larger sample sizes, a significant difference between the sexes would be demonstrated. By contrast, since growth is comparatively slow at Point Bailey, the absolute magnitude of any expected growth differences related to sex would be relatively small and hence masked by individual variability.

For all datasets, parameterisation of seasonal growth significantly improved the fit of GROTAG, indicating that seasonal variability in growth is significant for *N. fucicola* (Table 5.6 and 5.7). The estimates of seasonal growth from this study constitute the first for this species. Although a LRT suggested significant differences in the time of maximum growth (w) between sites, bootstrap estimates of w plotted in

Figure 5.5 b suggest that the LRT is overestimating the difference in the timing of peak growth. On balance therefore, peak growth in *N. fucicola* at both sites is estimated to occur during the summer maximum in water temperatures on the coast of Tasmania (e.g. Halpern *et al.* 2000). This suggests that there is a site independent effect of season on growth in *N. fucicola* post-settlement, as was determined in Chapter 4 for *Notolabrus* spp. in the early life stages. The mechanism affecting growth is worthy of further investigation, but is likely to include seasonal cycles in availability of food (Shepherd and Clarkson 2001; Denny and Schiel 2001) and temperature effects on metabolism, controlling resources allocated to growth throughout the year.

Sex-specific GROTAG analyses suggested a significant difference in measurement errors, with females being under measured by a mean of 3 mm, compared to less than 1 mm for males at Lord's Bluff. Differences in the order of several mm have been recorded in repeat measurements of *N. fucicola* (Ewing and Welsford unpub. data). Greater measurement errors for females have been detected in other studies using GROTAG (e.g. Simpfendorfer 2000), but a reason for greater difficulty in measuring females is difficult to determine. A possible explanation in this study is the outliers present in the dataset, high individual growth variability, and small sample size, shown to affect accurate estimation of measurement error in GROTAG (Francis and Mulligan 1998).

A significant difference in growth between the sexes at Lord's Bluff indicates that under conditions of rapid growth, female *N. fuciola* may grow significantly faster than males at the same site. Sex-specific growth rates have implications for the management of the fishery for *N. fucicola*. As the legal minimum size of *N. fucicola* is currently 30 cm, and in pre-recruitment size classes female growth rates are more

rapid than males (Figure 5.5), sex specific growth rates may result in differences in sex specific fishing mortality. However, as *N. fucicola* can be sexually mature at lengths of 12 cm (Patterson 2000), some individuals are likely to have spawned for 6-8 years before recruitment to the fishery at Lord's Bluff (Figure 5.1). This suggests that the minimum legal size limit is effectively protecting the reproductive output of the pre-recruit population of *N. fucicola.*

The potential for females to grow faster than males, as observed at Lord's Bluff, can be explained in the light of sex allocation theories (e.g. Charnov 1982; Warner 1991), assuming that individuals will attempt to maximise fitness in terms of reproductive output, by allocating resources to gamete production, or growth, or changing sex. As larger females are able to produce many more gametes than smaller females, and males of any size are likely to be able to produce oversupplies of sperm, greater growth for females produces greater potential for future fitness. Furthermore, Jones and Thompson (1983), studying the New Zealand protogynous hermaphrodite *N. celidotus* found that larger females were able to inhibit maturity in smaller females on the same reef. In *N. fucicola,* rapid growth may also be linked to future social dominance and increased fitness.

However, this exemplifies an apparent anomaly seen in the wrasses of the genus *Notolabrus*. *Notolabrus fucicola* co-occurs with and is so closely related to protogynous hermaphrodites *N. tetricus* and *N. celidotus* that hybrids occur in the wild (Ayling 1980). *Notolabrus fucicola* is the only confirmed gonochoristic species in the genus, or amongst any of the pseudolabrine wrasses (*sensu* Russell 1988), to which *Notolabrus* and 3 other temperate and sub-tropical genera belong (Russell 1988; Barrett 1995a, Denny and Schiel 2002). All the other pseudolabrine wrasses are protogynous hermaphrodites, with varying incidence of initial phase males (Jones

1980; Russell 1988; Barrett 1995a). Why do large *N. fucicola* females not change sex and exclude smaller males from mating opportunities? It may be that the fact that conditions allow rapid growth, such as Lord's Bluff, are rare or unpredictable in space and time. Hence making a choice to allocate resources to growth, to attain a large enough size to gain social dominance and change sex, at the expense of production of gametes, is too unreliable for *N. fucicola.* As seen in the broad range of spawning dates in settling wrasses on the east coast of Tasmania (Chapter 4), it may be females that can produce eggs for the longest period possible each spawning season that will be the fittest, by spreading risk against variability in the planktonic environment. Further study of the evolution and variability of reproductive strategies in temperate wrasses is a potentially rich source of examples for the refinement theories of sex allocation theories (Charnov 1982; Warner 1991), including determining how gonochorism and protogynous hermaphroditism coexist as stable reproductive strategies in this genus.

This study suggests that growth rates of *N. fucicola* vary significantly across small spatial scales. At Point Bailey, few individuals reach the minimum legal size limit of 30 cm until 10 years of age, while at Lord's Bluff they do so at least two years earlier (Figure 5.1). An equivalent conclusion is evident from the GROTAG estimates, suggesting that a 28 cm fish at Point Bailey will take more than 2 years on average to reach 30 cm, while a fish of similar size will reach the legal size within a year at Lord's Bluff (Figure 5.7). Hence relative yields and rates of replacement of recruited size and age classes are likely to be much lower at Point Bailey than Lord's Bluff, where length classes recruit at a greater rate.

Barrett (1999) using whole otoliths found no growth differences between several populations of *N. fucicola* in southeastern Tasmania, and used these findings

to support the hypothesis that temperate reef fish populations are not resource limited. The current study did not specifically address any hypothesis about resource limitation in temperate reef fishes, but has demonstrated clearly that growth rates can vary between populations at the scale of individual reefs. *Notolabrus fucicola* are site-attached once they settle out of the plankton, rarely having an ambit of more than 500 m on contiguous reef (Barrett 1995a; b; Welsford unpub. data), and rarely crossing soft bottom habitat if they are resident on smaller patch reef habitat (Barrett 1995a; b). Intuitively, it follows that if productivity varies between reefs, then the potential for growth of individual site-attached reef fish may be limited. Further study is advocated to determine the factors that influence growth at this scale, as a variety of factors have been cited in other temperate reef species where spatial variability is evident. These include habitat type (Barrett 1995 a; 1999; Gillanders 1997), conspecific competition and variation in juvenile recruitment (Jones 1980; 1984), and impacts of exploitation (Buxton 1993). However, as demonstrated here, the choice of growth model and the methods used to compare parameter estimates are critical to ensuring that differences in growth are detected, and if detected, are interpretable. We recommend the use of a combination of approaches, including growth models with biologically interpretable parameters, robust statistical tests such as LRTs, and plots of bootstrap parameters, as providing the greatest insight into the growth dynamics for fish species.

6. General Discussion

As outlined in the general introduction, despite their diversity, important ecological role, and basis in developing fisheries, southern temperate wrasses have rarely been studied in detail. The specific implications of the chapters above have already been discussed. However, the results of these individual chapters enable a broader discussion of phenotypic plasticity in the different life stages of *Notolabrus*, and the evolution of pseudolabrine wrasses and their diverse life strategies in temperate habitats in the southern hemisphere.

Pseudolabrine taxonomy

Techniques applied in Chapter 2 provide an example of the difficulties that can be faced generating the most basic of information i.e. the taxonomic identity of early life-history stages. As a group, the pseudolabrine genera provide little information when scrutinised by traditional methods of identifying fish larvae (i.e. head spination, pigmentation and meristics: Leis and Rennis 1983; Richards and Leis 1984; Neira *et al.* 1998). Previous studies also provided conflicting information on species identity and morphology. For example, only recently *Pseudolabrus japonicus* has been resolved into two species by *in situ* observations of spawning behaviour (Matsumoto *et al.* 1997) and morphological analyses combined with *post hoc* genetic analyses (Mabuchi and Nakabo 1997). However, all studies of larval development for this species are derived from data collected prior to this taxonomic revision (Mito 1962; Okiyama 1988; Kimura *et al.* 1998), and it is still unresolved as to which species developmental series apply. Similarly, in Chapter 2, genetic techniques provided the

only reliable identification of *Notolabrus* spp. and wrasses from other closely related genera.

As restriction fragment length polymorphism techniques are simple and relatively cheap, and also produce an amplified mtDNA fragment as a byproduct, the techniques described in chapter 2 would be suitable for screening large numbers of samples, in the instance of studies requiring the identification of planktonic larvae or eggs (e.g. Hare *et al.* 1994; Lindstrom 1999), as well as determining the phylogenetic relationships between pseudolabrine wrasses. This would have the potential to provide a unique opportunity to correlate contemporary patterns of social and reproductive behaviour with evolutionary relationships and timing of speciation, and provide insights into the evolution of temperate reef ecosystems.

Plasticity and environmental constraints in *Notolabrus* life-history parameters

As discussed in the general introduction, ecological variety reaches a maximum of expression in the diversity of species of wrasses found worldwide. Phenotypic plasticity, in the expression of characters such as growth between individuals in response to an individual's experience of the environment, rather than as a result of the evolution of previous generations *sensu* Warner (1991), is striking in the results above for *Notolabrus* spp.

Phenotypic plasticity is expressed in the early life-history stages of *Notolabrus* developing under a broad range of water temperatures. As shown in chapter 2, in the waters off the coast of Tasmania, it takes many days longer to reach developmental landmarks such as yolk sac absorption and the initiation of daily rings in the otoliths than for wrasses (Victor 1982). Evidently, data on developmental rates can not be

directly transferred from tropical to temperate taxa. This result has implications for other studies of temperate zone wrasses, such as *Semicossyphus pulcher* in California, where otolith microstructure is extensively used (e.g. Victor 1986b; Cowen 1991). If *S. pulcher* took a similar time to form the first increments in its otoliths, maximum planktonic larval duration (PLD) could be as high as 90 days, representing an increase of more than 15% of current estimates for this species (Victor 1986b). Further study is called for to determine the scale of effect of water temperature on development rates, how development is reflected in the otolith microstructures, and the relative importance of such effects to spawning and settlement date estimation.

Environmental temperature was also significantly correlated with seasonal timing of spawning and settlement in *N. fucicola*, described in detail in chapter 4. As described in the discussion of that chapter, due to the broad spawning period of adult *N. fucicola*, the likely experiences of individuals in any cohort in the first year of life is likely to be strikingly different. However, larval *N. fucicola* are evidently capable of racing through the larval stage in as little as 40 days, or spending more than twice that time in the plankton, and surviving to settle. Such variability is a common phenomenon in wrasses (Victor 1986a; b; Cowen 1991). Other studies utilising otolith measurement to infer size at settlement and larval growth show that delayed metamorphosis does affect survival in a non-labrid temperate reef fish (Shima and Findlay 2002), but has minimal effect in *Semicossyphus pulcher* (Cowen 1991) and Caribbean wrasses (Victor 1986c). A logical next step is to determine the cost of delayed metamorphosis or slow larval growth in *N. fucicola*. Such a study would use back calculated growth trajectories based on otolith increment widths. A prerequisite of such a study would be proper validation of the relationship between otolith size and body size, including a description of growth in *Notolabrus* larvae captured in the field.

Sampling of larvae could be conducted simultaneously with assessment of settlement to artificial habitats, and hence generate data on the relationship between larval supply and recruitment to the post-settlement population.

Post-settlement growth is evidently highly variable, through to the adult phase of *N. fucicola*, as detailed in chapter 5. Here again, temperature is correlated with the growth capacity of any individual, as shown in the significant seasonal component of growth detected in the length based models. The source of this seasonal variability is still hypothetical, but it is likely to be a combination of seasonal temperature variations limiting metabolism and affecting activity levels, allocation of energy to reproduction, and availability of food.

Improved understanding of individual reproductive physiology, and allocation of energy to gamete production throughout the spawning season by wrasse at different sites in different size classes, would further enhance our understanding of the life-history strategies used by temperate wrasses. For example, which individuals spawn when during the year? As hypothesised in chapter 4, larger female *N. fucicola* may have an advantage over smaller individuals. They may exclude smaller females from mating opportunities, as seen in *N. celidotus* (Jones 1980). Large fish may also be able to sustain spawning over a longer period, and therefore maximise their chances of producing larvae that will achieve the optimum combination of brief larval life and long growing season post-settlement in an unpredictable environment.

Studies of the energy budget of wrasses would enhance trophic models of temperate reef ecosystems, as they are important predators of invertebrates (Denny and Schiel 2001; Shepherd and Clarkson 2001). Shepherd and Clarkson in particular arguing that *N. tetricus* is a significant driver of South Australian reef ecosystem dynamics through its consumption of herbivores such as abalone, a claim worthy of

further investigation. Growth variability among individuals is also shown to be a characteristic of adult populations of wrasses in chapter 5. With the high level of size variation in settlement stage fishes, it may be that a component of this variability is a result of the timing of settlement of an individual, and its subsequent growth in the first year of life.

With the description of the characteristics of the settlement cohort at two sites over three years in chapter 4, an opportunity exists to assess the potential of using settlement as an index of recruitment to the adult population. The wrasse that settled in the 1998-1999 to 2000-2001 cohorts would now be available to the traps that were used to capture adults in chapter 5. Sampling the now 2+, 3+ and 4+ cohort on reef adjacent to the Bicheno and Recherche Bay artificial habitat sites, and inspecting the otoliths in the region corresponding to the first year of life, could show what characteristics of individuals cohort, at settlement, corresponds with those reaching adulthood. Hence it is possible to develop datasets to address questions on the relationships between early life stages and the adult population for *Notolabrus*, the influence of climate over longer time scales such as El Niño/La Niña, and the effect of the developing wrasse fishery on recruitment.

Evolution of temperate wrasse life histories

Because the centre of diversity for wrasses is tropical (Choat 1991), it is likely wrasses evolved in tropical systems and then subsequently moved into temperate regions. The ability to spawn all year, and vary the time spent in the plankton, as in the tropical wrasse *Thalassoma bifasciatum* (Thresher 1984; Victor 1986a), might be seen as a pre-adaptive characteristic for colonising temperate reefs, which as discussed in chapter 4 enables bet-hedging in the face of environmental variability.

Phylogenetic information would assist in determining whether pseudolabrine wrasses (including *Notolabrus* and other temperate genera) evolved in temperate habitats or when and where they arrived from tropical regions. This would enable hypotheses to be generated as to which behavioural and biological characteristics of pseudolabrines are specific responses to the temperate environment, or pre-adapted to enable them to succeed in colder waters.

The present range distributions of pseudolabrine wrasses also presents an intriguing natural experiment in mechanisms of speciation, overlaid with the diversity of social and reproductive patterns seen in the group. Many species have ranges delimited by oceanographic barriers, such as *Notolabrus celidotus*, geographically constrained by prevailing westerly currents and the lack of any habitat further east of its current range of coastal New Zealand (Russell 1988). Yet there are many examples of species groups that have overlapping ranges. In the introduction it was noted that *Notolabrus* spp. form hybrids in the wild, with *N. celidotus* x *N. fucicola* and *N. fucicola* x *N. inscriptus* hybrids reported in New Zealand (Ayling 1980), and *N. fucicola* x *N. tetricus* in south-eastern Australia (Gomon *et al.* 1994). It is particularly intriguing that *N. fucicola* is the one linking species in this complex, with a range that extends across both sides of the Tasman Sea, and is also the only gonochoristic species. There is also evidence that *N. fucicola* may be derived from a protogynous species, based on the presence of a structure interpreted as a residual ovarian lumen in the gonads of male *N. fucicola* (Barrett 1995a; Denny and Schiel 2002).. It is important to determine the evolutionary history of this group and how may this situation arisen. Is it the product of allopatric speciation, or could it be an example of sympatric speciation as has been hypothesised for cichlids in the lakes of Africa

(Dieckmann and Doebell 1999; Higashi *et al.* 1999; Kirkpatrick 2000)? What is maintaining the boundaries between them now? How are populations of *N. fucicola* in New Zealand related to those in southeastern Australia?

Development of models explaining the evolution of the diversity of sex change and social systems, and their interplay with other aspect of life histories of fishes have been particularly insightful when such complex phenomena are studied in coral reef fishes. For example, defensibility of resources, related to distribution of food and mates, and the density of competitors influences the incidence of territoriality of terminal phase males, and fitness of 'sneaker' initial phase males in *Thalassoma bifasciatum.* If resources are too widespread, or competitors too numerous, maintaining a high quality territory is too taxing, leading to the apparently counterintuitive observation that the 'best' territories are undefended (Warner 1991). Barrett (1995a) hypothesised that as *N. fucicola* is often found foraging in the near intertidal, and hence territorial behaviour based on food resources was meaningless for a species where territories disappear at low tide. The results of chapter 5, including the fact that *N. fucicola* females grow faster than males under conditions of fast growth, but that growth is highly variable between individuals and reefs suggests a complementary hypothesis. That is that under conditions of uncertain access to food resources, gaining a size advantage to exclude other individuals from a territory or from access to mates is too difficult. So *N. fucicola* may have abandoned sex-changing behaviour in favour of gonochorism.

Comparisons of the life- history parameters of *Notolabrus* with those of other common reef species in Tasmania, such as the morwongs (Cheilodactylidae), is also likely to provide insight into the selective pressures driving life-history strategies in temperate habitats. Morwongs appear to have strongly contrasting strategies to

wrasses. For example, the banded morwong (*Cheilodactylus spectabilis*) are known to live to in excess of 70 years in Tasmanian waters, males grow to larger sizes than females, sexual maturity occurs in excess of 10 years of age, and the spawning season each year is brief (Murphy and Lyle 1998). Morwongs larvae spend many months in the plankton, travelling long distances into offshore waters, and then return as a 'paper fish' stage, an actively swimming, large (5+ cm) post-larva, to settle on the reef (Bruce *et al.* 2001; Jordan 2001a;b). The population dynamics of wrasses and morwongs are likely to be very different, but both are successful in that large populations of species from both groups persist on reefs in Tasmania. From the point of view of managing the fisheries of both groups, it would be useful to know if recruitment is more variable in morwongs than wrasses, and under which conditions each life strategies is most successful.

Evidently, testing such models requires much more information than is currently available on such factors as habitat preferences, population dynamics, phylogeny, resource allocation, and social interactions within and between species. Importantly this kind of study also has application in developing adequate models for assessing and managing the impact of increasing fishing based on temperate reef species. The techniques developed in this thesis provide a platform on which such studies can be built. The results of the studies detailed above considerably broaden the picture as to factors influencing the life-history strategies of southern hemisphere temperate wrasses, and also provides information on the ecology and biology of *N. fucicola* which may enhance scientific management of the wrasse fishery in south-eastern Australia. Hopefully it also constitutes an argument for continued enquiry into the ecology of temperate reef fishes.

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