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The effect of parasitism by a blood-feeding isopod on the otolith chemistry of host fish

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Abstract. Otolith chemistry is widely used to discriminate fish stocks or populations, although many of the factors that determine trace-element concentrations within the otolith remain poorly understood. We investigated the effect of a blood-feeding isopod ectoparasite, *Ceratothoa* sp., on the otolith chemistry of yellowtail scad, *Trachurus novaezelandiae*. We sampled 65 fish from three subpopulations of *T. novaezelandiae* from Jervis Bay in south-eastern Australia, and used laser ablation (LA)–inductivelycoupled plasma mass spectrometry (ICPMS) to measure otolith lithium (Li) : calcium (Ca), magnesium (Mg) : Ca, strontium (Sr) : Ca and barium (Ba) : Ca from four consecutive summer and winter growth bands. Otoliths of parasitised fish were characterised by significantly lower Li : Ca and Mg : Ca, and higher Sr : Ca, than those of unparasitised individuals from the same subpopulation. The consistency of trends in otolith chemistry across ablation points and among subpopulations suggests that there is a consistent physiological mechanism through which *Ceratothoa* parasites affect the otolith chemistry of infected individuals. It is likely that a range of physical, metabolic, chemical and behavioural processes act in concert to influence the otolith chemistry of parasitised fish. Given the ubiquitous distribution of parasites in the marine environment, differential rates of parasitism among fish stocks, populations or migratory contingents may be an important but unappreciated factor driving stock- or population-based differences in otolith chemistry.

Additional keywords: Carangidae, Cymothoidae, ectoparasite, tongue biter.

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Introduction

Otolith chemistry is now widely used to discriminate fish stocks or populations. Trace elements are continually and permanently incorporated into the calcium carbonate matrix of fish otoliths (ear-stones) as they grow (Campana 1999). The concentration of elements within an otolith is influenced by a suite of environmental factors – most notably ambient seawater concentrations, temperature and salinity – so differences in otolith chemistry are used to infer that individuals have experienced different environmental histories, and to discriminate among populations or stocks of fish that have remained largely geographically distinct over a given period (Campana *et al.* 1999; Elsdon *et al.* 2008). Otolith chemistry techniques can provide insights into the population, and structure of marine fish at finer spatial scales than has previously been achieved using genetic or tagging techniques (Campana *et al.* 1999; Campana and Thorrold 2001; Elsdon and Gillanders 2005; Elsdon *et al.* 2008). Despite the large number of studies reporting significant differences in otolith chemistry among fish groups sampled (Thresher 1999), many of the factors that determine trace-element concentrations within the otolith remain poorly understood (Campana 1999; Elsdon *et al.* 2008).

In addition to environmental factors, physiology has been identified as an important determinant of otolith chemistry (Kalish 1989, 1991; Thresher 1999). Physiological factors are thought to influence otolith chemistry via changes in the protein composition of blood plasma (Kalish 1991) because trace elements that are ultimately incorporated into the otolith are found first within the blood plasma. The degree to which elements are bound as molecules *versus* free as ions within the blood plasma depends on the plasma protein composition; proteins vary in their binding potential and in their affinity for particular elements or ions, so changes in plasma protein composition can alter both the overall and relative abundances of free trace elements within the plasma (Kalish 1989, 1991). For example, seasonal breeding in the bearded rock cod, *Pseudophycis barbatus*, has been linked to changes in the volume and composition of proteins in the blood plasma that cause preferential binding of Ca^{2+} over Sr^{2+} and a subsequent

decrease in the relative abundance of free Ca^{2+} within the plasma (Kalish 1991). In fish, chemical changes in the blood plasma are mirrored by changes in the chemical composition of the endolymph, the aqueous substance that bathes the otolith within the ear canal and from which the otolith is precipitated (Kalish 1989, 1991; Payan *et al.* 2004). The final elemental composition of the otolith is dependent on the chemical composition of the endolymph (Kalish 1989; Campana 1999; Takagi 2002; Payan *et al.* 2004), although some further elemental discrimination appears to occur during precipitation (Kalish 1989; Campana 1999). Links between physiology and otolith chemistry have been reported for a range of physiological processes, including growth rate (Sadovy and Severin 1994; Martin and Thorrold 2005), stress (Townsend *et al.* 1989; Kalish 1992; Payan *et al.* 2004) and life-history events including metamorphosis (Otake *et al.* 1994; Tzeng *et al.* 1997) and reproduction (Kalish 1989; Friedland *et al.* 1998); however, other potential physiological determinants of otolith chemistry remain unexplored. A greater understanding of the range of physiological factors affecting otolith chemistry may elucidate the mechanisms that drive otolith trace-element incorporation and assist in the validation and accurate interpretation of otolith-chemistry studies (Thresher 1999).

In the present paper, we investigate the potential effect of an isopod fish parasite, *Ceratothoa* sp., on the otolith chemistry of *T. novaezelandiae*. Most fish, from freshwater to coastal and deep-ocean ecosystems are parasitised to some degree, and many carry multiple parasites across a range of species (Lester 1990; Bunkley-Williams and Williams 1998). Numerous fish parasites, including *Ceratothoa* spp., are known to feed directly on blood or blood components of host fish (Adlard and Lester 1995; Bunkley-Williams and Williams 1998) and in doing so, they may alter the blood plasma through the injection of anticoagulants or other compounds, via effects on physiological processes such as nutrition, growth and condition, or by eliciting stress or immunological responses that alter blood proteins (Horton and Okamura 2003). Such changes are likely to influence the otolith chemistry of the host fish. We focus on the effects of *Ceratothoa* parasites on otolith concentrations of Li, Mg, Sr and Ba, elements that are among the most common discriminators of fish stocks and populations published in the literature.

Materials and methods

Study species

Ceratothoa parasites (Crustacea: Isopoda: Cymothoidae), also known as 'tongue biters', are blood-feeding ectoparasites that attach to the mouth cavity and gill filaments of host fish (Adlard and Lester 1995; Bunkley-Williams and Williams 1998). *Ceratothoa* parasites have inshore coastal distributions (Hale 1926; Lanzing and O'Connor 1975; Bunkley-Williams and Williams 1998), are long lived, and form permanent associations with their host fish (Bunkley-Williams and Williams 1998). *Ceratothoa* spp. have a free-swimming juvenile phase and mature on infection of a suitable host species. *Ceratothoa* parasites generally form stable protandrous pairs, with a large female inhabiting the mouth cavity and a smaller male attaching to the gill rakers (Maxwell 1982; Bunkley-Williams and Williams 1998). The taxonomy of Australian species of *Ceratathoa* is the subject of ongoing research. Given the uncertainty surrounding the taxonomy of these species, we have identified parasites observed in the present study only to genus level, with assistance from researchers at the Australian Museum. We consider it most likely that the species observed in our study is *Ceratathoa* cf. *imbricata* (Fabricius, 1775) because this species has previously been reported from coastal south-eastern Australian waters on a range of host species, including *T. novaezelandiae* and other trachurid and pelagic fish (Hale 1926; Lanzing and O'Connor 1975; Maxwell 1982; Perera 1993; Hayward *et al.* 1998). Parasites of this species range in age up to 9 years and in size from ~15 to 50 mm for females and ~5 to 25 mm for males (Maxwell 1982). Figure 1 shows a male and female pair of *Ceratothoa* parasites and the location of the larger female parasite in the mouth cavity of the host fish.

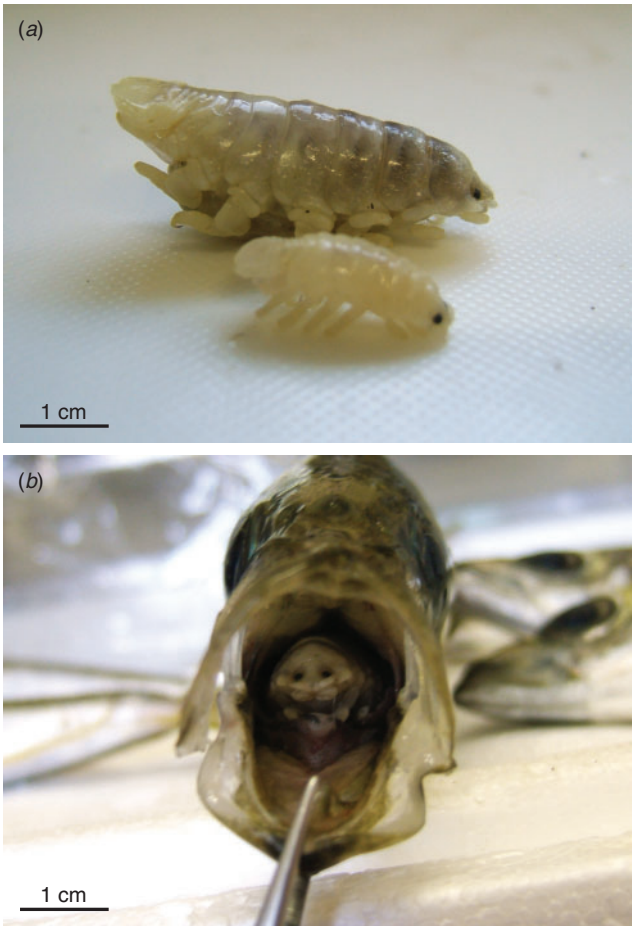

Fig. 1. (*a*) *Ceratothoa* parasites female (behind) and male (in front) pair and (*b*) location of female parasite in mouth cavity of *Trachurus novaezelandiae*.

The host species examined in the present study, *T. novaezelandiae*, is a small pelagic fish common in the coastal waters of southern Australia and New Zealand (Suda *et al.* 1995; Stewart and Ferrell 2001). We have previously conducted a study into the otolith chemistry of *T. novaezelandiae* from Jervis Bay and adjacent coastal waters in south-eastern Australia (Fig. 2). Otolith-chemistry signatures based on Li : Ca, Mg : Ca, Sr : Ca

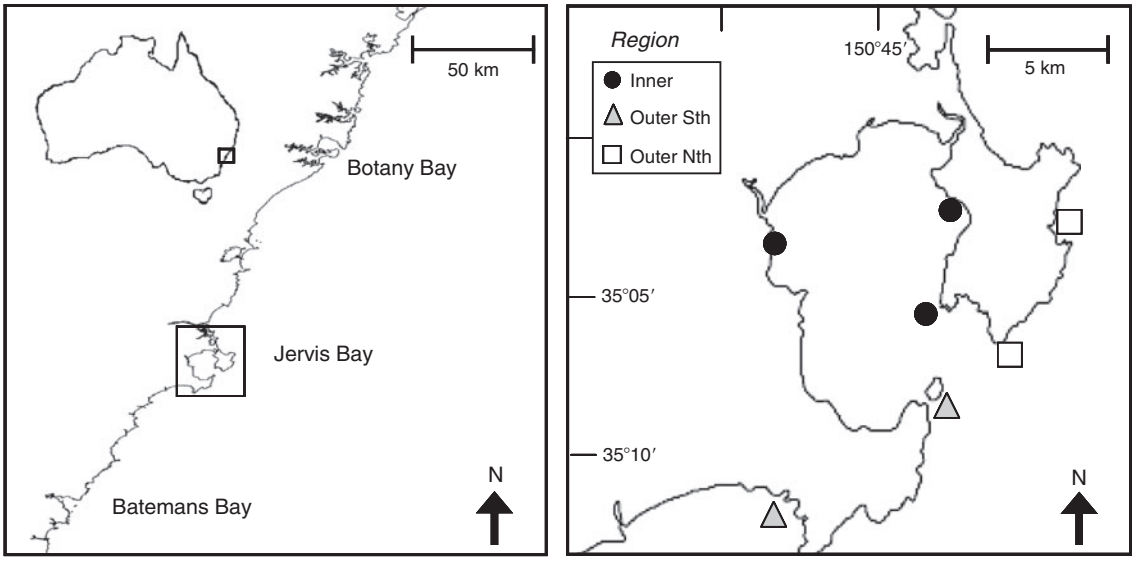

Fig. 2. Location of Jervis Bay and sampling sites in the Inner, Outer Southern and Outer Northern regions of the bay. All sampling sites were on or near subtidal reef habitat.

and Ba : Ca examined in that study revealed distinct groupings of *T. novaezelandiae* from the 'Inner', 'Outer Southern' and 'Outer Northern' regions of Jervis Bay (Fig. 2; E. C. Heagney, B. M. Gillanders and I. M. Suthers, unpubl. data) and suggested that regional groups represented distinct subpopulations or migratory contingents within the study area. We also identified significant ($P < 0.0005$) differences in the prevalence of *Ceratothoa* parasites among regional groups. The proportion of captured individuals with *Ceratothoa* sp. present in the mouth cavity was higher for the Inner and Outer Southern subpopulations (~70–80% parasitised) and lower for the Outer Northern subpopulation (~20% parasitised). We subsampled individuals from each population to determine the effect of the presence/absence of *Ceratothoa* parasites on otolith elemental concentrations.

Our subsample included 37 parasitised fish (23, 11 and 3 from the Inner, Outer Southern and Outer Northern regions, respectively) and 28 unparasitised fish (7, 7 and 14 from the Inner, Outer Southern and Outer Northern regions, respectively). All parasitised fish observed in our study had one large female parasite ~30–40 mm in length present in the mouth cavity; 95% of parasitised fish also had a smaller male parasite ~10–15 mm in length present in the gill rakers. This observation is consistent with Lanzing and O'Connor (1975), who reported that it was uncommon to find more than two *C. imbricata* parasites (one male and one female) on any individual host fish. The absence of a male parasite from a small number of infected individuals in our study may relate to these smaller parasites becoming dislodged when fish were submerged in ice slurry, rather than reflecting a true 'absence' of male parasites.

Otolith preparation, ageing and analysis

Fish were measured (fork length) and sagittal otoliths were extracted on the day of capture with plastic forceps. One otolith from each fish was washed in distilled water, air-dried and stored in a paper envelope. A subsample of 67 otoliths from the 135 collected was taken to include 17–30 otoliths from each sample region. These otoliths were embedded in a two-part epoxy resin (Epofix, Struers, Ballerup, Denmark) and a 300–400-μm section was cut through the core of each otolith with a low-speed diamond saw. Sections were affixed to glass slides with indium-spiked glue (Crystal Bond, SPI, West Chester, PA, USA), with three sections randomly assigned to each slide. Sections were polished using 9-μm and 3-μm aluminium oxide lapping film.

Otoliths were aged under a high-powered imaging microscope, according to the ageing technique outlined in Stewart *et al.* (1999) and Stewart and Ferrell (2001), using a reference collection of 100 *T. novaezelandiae* otoliths held by the New South Wales Department of Primary Industries. Prior to the chemical analysis, otoliths were re-polished using 3-μm aluminium oxide lapping film and ultrapure water, sonicated in ultrapure water for 1 min to remove any surface contamination, dried overnight under a fume hood to prevent dust settling on the sectioned surfaces, and stored in air-tight plastic bags. Chemical analyses were conducted using a laser microprobe (New Wave Nd Yag 213 UV) and inductively coupled plasma mass spectrometer (Agilent 7500cs) at the University of Adelaide. Laser ablations were performed at 75% power using a 30-μm spot laser, with four ablations made on each otolith as follows: one on the outermost opaque seasonal growth band and one on each of the three preceding translucent and opaque bands. Taking into account both the seasonality of banding and the time lag observed in the formation of annuli for this species (Stewart *et al.* 1999), the four otolith regions sampled correspond to material laid down in two austral summers (Summer 2004–2005 and Summer 2003–2004) and two austral winters (Winter 2004 and Winter 2003). To minimise potential effects from heterogeneous elemental concentrations across the otolith surface (Thresher 1999), ablation series were conducted, where possible, in one of two loci, namely, along the ventral edge of the sulcus or at the outer edge of the otolith. The specific locus sampled on each otolith was chosen to avoid any visible pits or

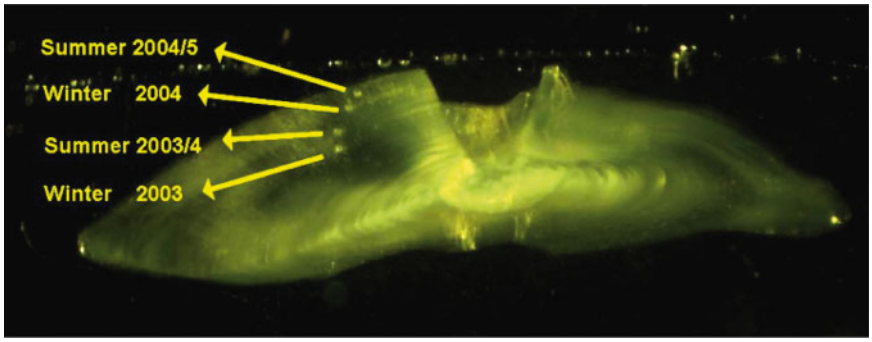

Fig. 3. *Trachurus novaezelandiae* otolith, showing location of ablation pits on seasonal growth bands.

cracks in the otolith surface, because these features may be associated with an increased risk of contamination (Thresher 1999). The location of each ablation point was subsequently verified and photographed using a high-powered imaging microscope to ensure that ablation points were located wholly within the relevant seasonal growth band. An example of the ablation pattern on otolith growth bands is provided in Fig. 3. The chamber was purged for 20–30 s between ablations, and a reference standard (National Institute of Standards and Technology, NIST 612) was ablated after every 12 sample ablations to correct for machine drift.

Six elements were sampled for potential inclusion in otolith-chemistry signatures (Li, sodium (Na), Mg, zinc (Zn), Sr and Ba) with detection limits for each element calculated from background sampling of chamber gases for 30 s before each ablation. Four elements were selected for final analysis (Li, Mg, Sr and Ba) because these were consistently above the detection limits and are considered to be stable and reproducible (Campana 1999; Thresher 1999; Campana *et al.* 2000), and because they are among the most common discriminators of fish stocks and populations published in the literature. Elemental concentrations in parts per million (ppm) were converted to molar concentrations and expressed as a ratio to ^{44}Ca (mmol element per mol Ca).

Statistical analyses

Otolith elemental concentrations were 4th-root transformed to improve heterogeneity of variance identified among regional groups. Repeated-measures ANCOVA was used to investigate potential effects of age, fork length and growth rates (Age × Fork length interaction term) on otolith elemental concentrations. Age was found to have a significant effect on Sr : Ca ($P = 0.003$) and Ba : Ca ($P = 0.005$), so concentrations of these two elements were adjusted to account for the slope of the regression by using the technique outlined in Bergenius *et al.* (2005). Insufficient data were available to establish correlations between age and elemental concentrations for fish younger than 3 years or older than 5 years, so only fish within the 3–5-year-old age bracket were included in subsequent analyses ($n = 65$). For each 3–5-year-old fish, we constructed a multi-elemental signature, comprising the (transformed) concentrations of Li : Ca, Mg : Ca, (age-adjusted) Sr : Ca and (age adjusted) Ba : Ca from each of the four seasonal ablation points on each otolith.

We conducted two-factor ANCOVA with random factor 'Region' and fixed factor 'Parasite' (presence *v.* absence) to investigate potential effects of *Ceratothoa* parasites on the growth rates of *T. novaezelandiae* (dependent variable fork length; covariate age). We used two-factor repeated-measures ANOVA with factors 'Region' and 'Parasite' to investigate the effect of *Ceratothoa* parasites on otolith Li : Ca, Mg : Ca, Sr : Ca and Ba : Ca, and repeated-measures MANOVA to investigate effects on otolith-chemistry signatures averaged across all four ablation points. For ANOVA, we used Huynh–Feldt corrected values to account for non-sphericity of data indicated by significant Maulchy's tests; for MANOVA we used the Wilk's lambda *F*-statistic because it is robust against heterogenous variances (Tabachnick and Fidell 1983). Type III sum of squares was used in ANCOVA and ANOVA to account for missing data. All analyses listed above were conducted using SPSS (Version 14.0, SPSS Inc., Chicago, Illinois, USA).

We used cluster analysis to compare the relative scale of regional effects *versus* effects of *Ceratothoa* parasites on otolith chemistry, comparing otolith-chemistry signatures across six Region × Parasite combinations (*Ceratothoa* present/absent for each of three sample regions). Cluster analysis was based on a Euclidean distance similarity matrix and was performed using PRIMER (Version 6, PRIMER-E, Plymouth, UK). We performed discriminant function analysis (DFA) on regional otolith-chemistry signatures of all fish in SYSTAT (Version 10, SPSS Inc.) by using a backward stepwise regression to exclude extraneous variables, and constructed reclassification matrices using jack-knifed cross-validation. We repeated DFA using data from fish with *Ceratothoa* present *versus* absent separately and compared the success rates from reclassification matrices in each case, so as to infer how regional or population-based differences in parasitism might affect the interpretation of results from otolith-chemistry studies.

Results

We did not detect any difference in growth between parasitised and unparasitised individuals within each region (ANCOVA, $P = 0.99$). Univariate analyses identified significant differences in otolith Li : Ca, Mg : Ca and Sr : Ca between parasitised and unparasitised individuals that were consistent across all three sample regions (Table 1). *T. novaezelandiae* infected with *Ceratothoa* parasites had significantly lower Li : Ca and Mg : Ca, and higher Sr : Ca than di unparasitised individuals (Fig. 4).

Table 1. Mean squares (MS) and significance levels from repeated-measures ANOVA/*F*-values and significance levels from MANOVA comparing effects of sample region, season and presence/absence of *Ceratothoa* parasites on elemental concentrations from four ablation points on *Trachurus novaezelandiae* otoliths

d.f., degrees of freedom for univariate tests. For MANOVA, Wilks' lambda *F*-statistic is shown. For MS Li, MS Mg, MS Sr and MS Ba within subject effects, Huynh–Feldt corrected values are shown.*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$

Factor	d.f.	MS Li	MS Mg	MS Sr	MS Ba	MANOVA
Between-subject effects						
Region	2	0.008	0.011*	0.060***	0.151***	11.73***
Parasite	1	0.038***	0.015*	0.029*	0.023	3.947**
Region × Parasite	2	0.001	0.002	0.004	0.007	0.658
Error	59	0.003	0.003	0.006	0.007	
Within-subject effects						
Season	3	0.000	0.005	0.028***	0.002	6.458***
Season × Region	6	0.001	0.005	0.002	0.003	1.388
Season × Parasite	3	0.000	0.002	0.000	0.001	0.228
Seas × Reg × Para	6	0.000	0.001	0.001	0.003	0.589
Error	177	0.001	0.003	0.001	0.002	

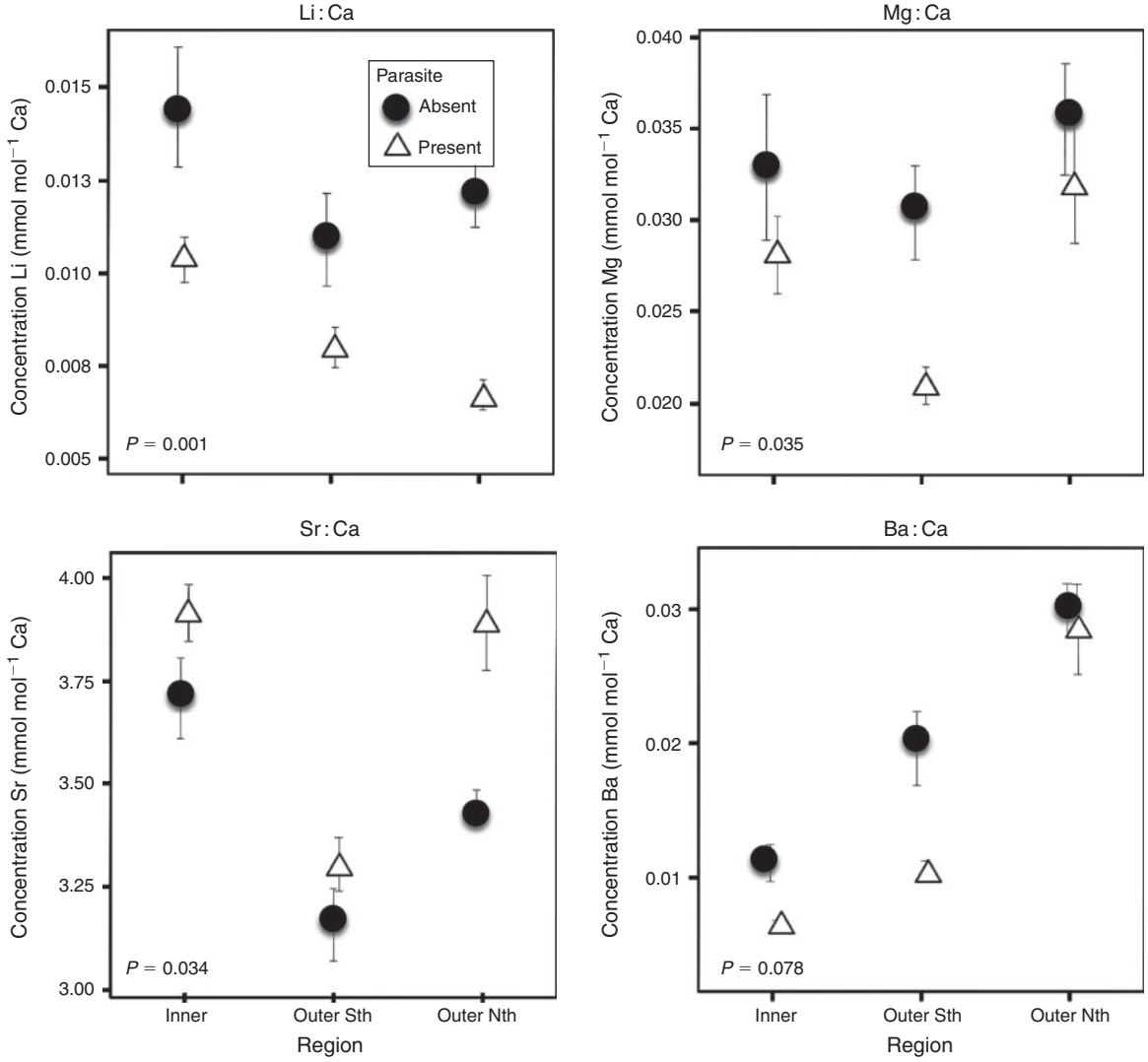

Fig. 4. Mean (±s.e.) concentration of lithium (Li) : calcium (Ca), magnesium (Mg) : Ca, strontium (Sr) : Ca and barium (Ba) : Ca in otoliths of *Trachurus novaezelandiae* with and without *Ceratothoa* parasites from each sample region. Concentrations are averaged across all four seasonal ablation points sampled. Sr and Ba concentrations were adjusted for age. *P*-values indicate results for 'Parasite' term from repeated-measures ANOVA (see Table 2).

Differences in otolith elemental concentrations observed in parasitised *v.* unparasitised fish within each region were consistent across all four ablation points sampled (Fig. 5). Ba : Ca was also significantly reduced in parasitised individuals in two of three sample regions; however, given the inconsistency of this result in the Outer Northern region, ANOVA comparing Ba : Ca in parasitised *versus* unparasitised individuals yielded a marginally non-significant result overall ($P = 0.078$; Figs 4, 5).

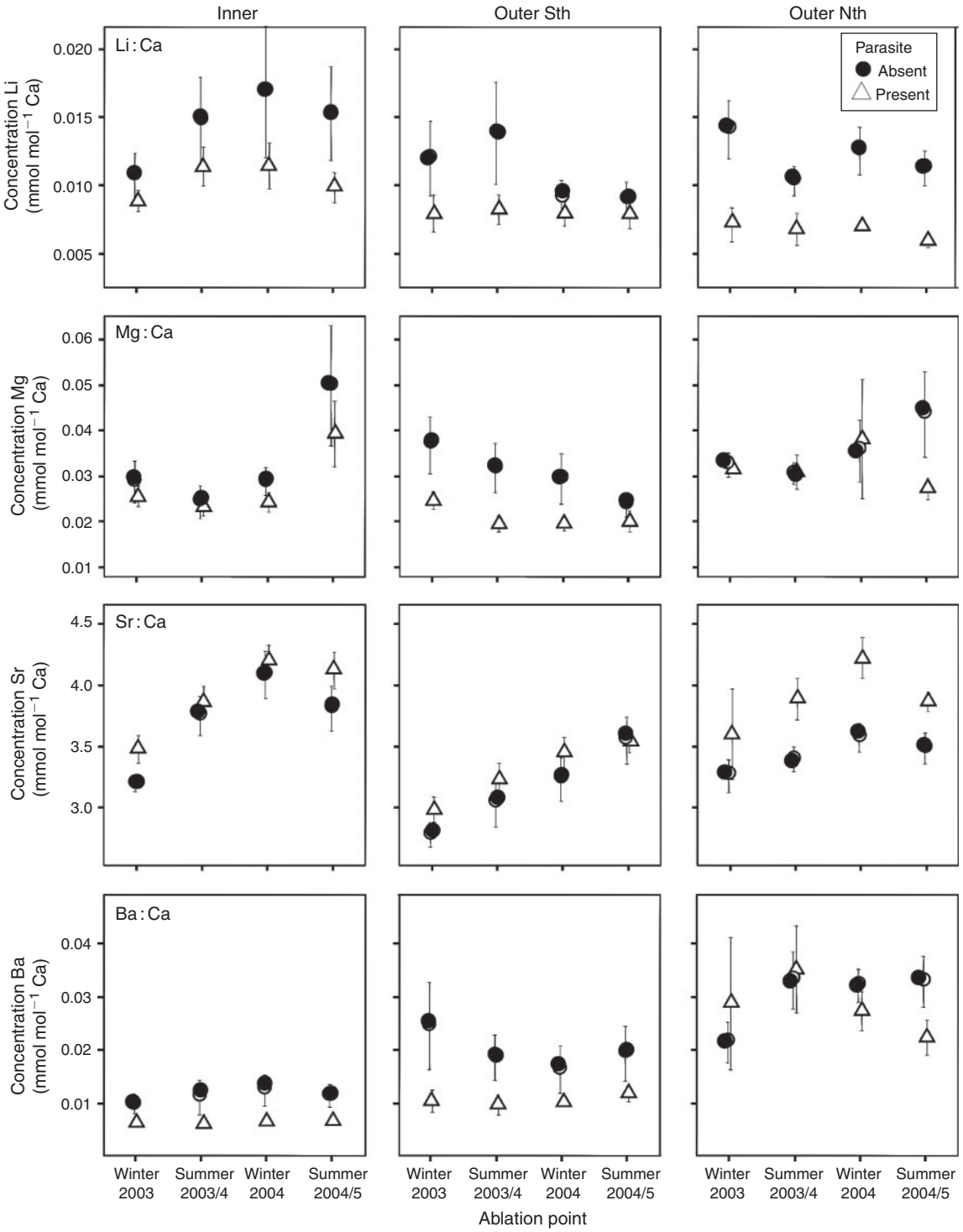

Fig. 5. Mean (±s.e.) seasonal concentration of lithium (Li) : calcium (Ca), magnesium (Mg) : Ca, strontium (Sr) : Ca and barium (Ba) : Ca in otoliths of *Trachurus novaezelandiae* with and without *Ceratothoa* parasites from each sample region. Concentrations are mean values from each seasonal ablation point. Sr and Ba concentrations were adjusted for age.

MANOVA identified significant differences in *T. novaezelandiae* otolith-chemistry signatures among regions ($P < 0.0001$) and between parasitised and unparasitised individuals ($P = 0.007$; Table 1). Cluster analysis grouped our six Region × Parasite combinations by region, indicating that the effect of *Ceratothoa* parasites on otolith chemistry was smaller than the observed regional differences (Fig. 6). DFA separating *T. novaezelandiae* into regional groups on the basis of otolith chemistry had the highest reclassification success rate (95%) when analyses used only data relating to parasitised fish (Table 2, Fig. 7). This higher reclassification success was achieved despite a dramatic reduction in the number of fish included in the analysis ($n = 37$ parasitised fish *v.* $n = 65$ in the overall analysis).

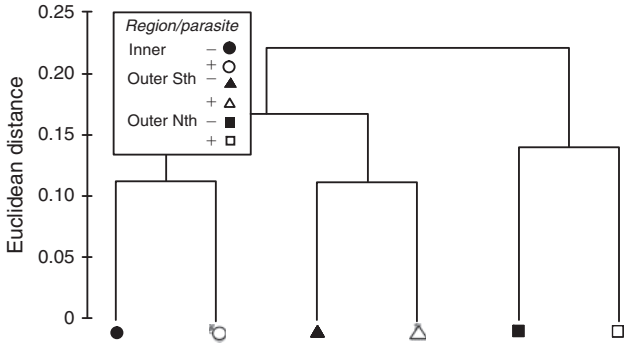

Fig. 6. Cluster diagram, showing groupings of *Trachurus novaezelandiae* based on region of capture and presence (+) or absence (−) of *Ceratothoa* parasites. Cluster analysis was performed on multi-season, multi-elemental otolith-chemistry signatures (Li : Ca, Mg : Ca, Sr : Ca and Ba : Ca from each of four seasonal ablation points).

Discussion

Ceratothoa parasites had a significant effect on the otolith chemistry of *T. novaezelandiae*, with otoliths of parasitised fish characterised by significantly lower Li : Ca and Mg : Ca, and higher Sr : Ca than those of unparasitised individuals from the same region. Ba : Ca was also lower in the otoliths of parasitised fish (cv. those of unparasitised fish) from two of three sample regions and for two of four ablation points in the remaining (Outer Northern) region. Although ANOVA comparing Ba : Ca in parasitised *versus* unparasitised fish yielded a marginally non-significant result overall, this may relate to the small number of parasitised fish available for subsampling from the Outer Northern region ($n = 3$) or to parasitised individuals from the Outer Northern region having been infected relatively recently because of their distribution along the exposed coastline to the north of Jervis Bay, which is unlikely to overlap extensively with the inshore distribution of *C. imbricata.*

The direction and magnitude of differences in otolith Li : Ca, Mg : Ca and Sr : Ca between parasitised and unparasitised fish were similar across each of the four seasonal ablation points sampled, indicating that otolith elemental concentrations of host fish had been affected by *Ceratothoa* parasites over the preceding 2-year period, a finding that is consistent with reports that *C. imbricata* is a long-lived parasite that forms permanent associations with its host fish (Maxwell 1982; Bunkley-Williams and Williams 1998). The consistency of trends in otolith chemistry across ablation points and among sample regions also points to a consistent physiological mechanism through which *Ceratothoa* parasites have an impact on the otolith chemistry of infected individuals.

There are several plausible pathways through which *Ceratothoa* and other cymothoid parasites might affect otolith chemistry, including physical, metabolic, chemical and behavioural processes. Physical effects associated with the presence

Table 2. Re-classification matrices from discriminant function analysis (DFA), showing agreement between region of capture and region predicted by multi-season otolith-chemistry signatures for all *Trachurus novaezelandiae*, for individuals with *Ceratothoa* absent and for individuals with *Ceratothoa* present

Bold indicates that individuals are correctly reclassified into their region of capture

Region of capture	Region predicted by otolith-chemistry signature			
	Inner, *n*	Outer Southern, *n*	Outer Northern, *n*	Agreement (%)
All fish				
Inner ($n = 30$)	**26**	4	0	87
Outer Southern ($n = 18$)	2	**14**	2	78
Outer Northern ($n = 17$)	1	1	**15**	88
Overall ($n = 65$)				**85**
Parasite absent				
Inner ($n = 7$)	**5**	2	0	71
Outer Southern ($n = 7$)	1	**4**	0	57
Outer Northern ($n = 14$)	0	1	**13**	93
Overall ($n = 28$)				**79**
Parasite present				
Inner ($n = 23$)	**21**	2	0	91
Outer Southern ($n = 11$)	0	**11**	0	100
Outer Northern ($n = 3$)	0	0	**3**	100
Overall ($n = 37$)				**95**

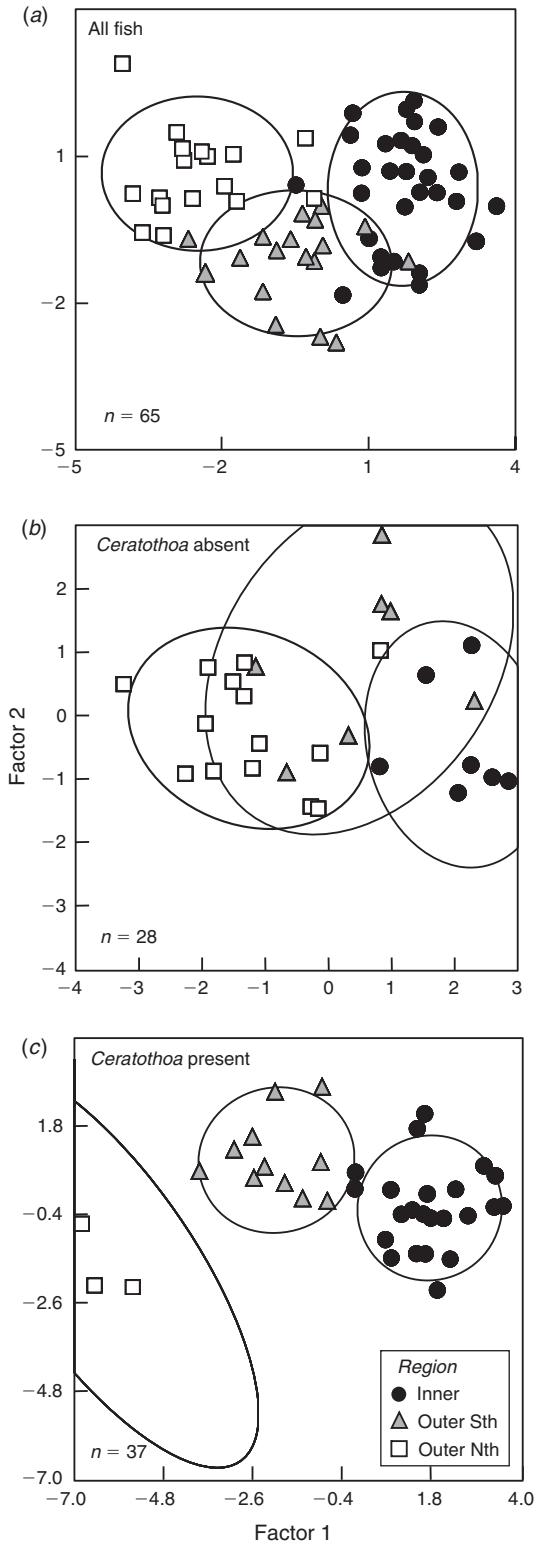

Fig. 7. Discriminant function scores and 95% confidence ellipses around data points for otolith-chemistry signatures of *Trachurus novaezelandiae* caught in each sample region for (*a*) all fish, (*b*) individuals with *Ceratothoa* absent and (*c*) individuals with *Ceratothoa* present. Factor 1 was correlated with barium (Ba) (−) and lithium (Li) (+); Factor 2 was correlated with magnesium (Mg) (−).

of a large cymothoid parasite in the gill chamber or mouth cavity include the obstruction of water flow over the gills (Adlard and Lester 1995), reduced gill function from lesions on gill filaments and, in severe cases, the loss of some or most gill filaments (Bowman 1960; Adlard and Lester 1995). Incomplete gill function may reduce elemental discrimination and has previously been linked to elevated otolith Sr : Ca, as observed for parasitised fish in our study (Kalish 1991). Metabolic effects of cymothoids include reduced fish growth, fecundity and condition (Lanzing and O'Connor 1975; Adlard and Lester 1995; Sievers *et al.* 1996) – all of which have been linked to changes in otolith chemistry (e.g. Kalish 1991; Sadovy and Severin 1994; Martin and Thorrold 2005).

Chemical changes observed in the blood of fish infected by cymothoid parasites include anaemia (Adlard and Lester 1995; Horton and Okamura 2003) and associated reductions in blood erythrocyte, haematocrit and haemoglobin concentrations, increased eosinophilic granulocytes associated with inflammation, increased neutrophilic granulocytes characteristic of stress, and increased leucocyte levels associated with an immunological response to disease or infection (Horton and Okamura 2003). Cymothoids may also inject anti-coagulants or other compounds directly onto the blood. All these changes in the composition of blood protein are likely to affect the binding potential of the blood plasma and its affinity for particular ions. Although specific effects on otolith chemistry remain unknown for most blood compounds, changes in blood proteins associated with stress have previously been linked to increased otolith Sr : Ca, consistent with observations in our study (Kalish 1991). Fish infected by cymothoid parasites may also display different schooling patterns, habitat selections or migration behaviours compared with unparasitised fish (Guthrie and Kroger 1974; Lester 1990) – behavioural changes that are likely to have a major impact on the environmental history, and hence the otolith-chemistry signature, of host fish.

It is most likely that some or all of the processes described above act in concert to influence the otolith chemistry of parasitised fish. In our study, we found no effect of presence *versus* absence of *Ceratothoa* parasites on the growth rates or migration patterns *T. novaezelandiae* (the latter was consistent within each regional subpopulation – E. C. Heagney, B. M. Gillanders and I. M. Suthers, unpubl. data). We, therefore, consider it most likely that *Ceratothoa* parasites affected otolith chemistry by inducing changes in condition and fecundity and/or through physical and chemical mechanisms. Although *Ceratothoa* spp. and other cymothoids are among the largest fish parasites (Bunkley-Williams and Williams 1998), other parasites also have the potential to act on otolith chemistry pathways through any or all of the physical, chemical, metabolic and behavioural mechanisms discussed above. In particular, metabolic changes in fish condition and chemical changes in the blood plasma related to the immunological response of the host fish to parasitism are likely to be common across a broad range of parasites.

Parasites have often been used as biological tags and differences in parasite loads or the composition of parasite assemblages have long been used to delineate fish stocks, population structure and migration patterns (Lester 1990; Cuyás *et al.* 2004). Over the past 30 years, otolith-chemistry studies have

fulfilled similar functions. Our results imply that differential rates of parasitism among stocks, populations or migratory contingents may be one of the factors driving differences in otolith chemistry. Other trends in otolith chemistry, such as temperature effects on otolith Sr : Ca (e.g. Bath *et al.* 2000; Elsdon and Gillanders 2004; Martin *et al.* 2004), may also be linked to parasite loads, because parasite abundance and species richness are strongly correlated with temperature for many host species (Hayward *et al.* 1998). Otolith techniques may be able to capture stock- or population-based differences in parasite loads without some of the problems associated with parasitological studies, including ongoing taxonomic confusion and the need for specialist taxonomic expertise (MacKenzie and Abaunza 1998), intensive sampling requirements for quantifying parasite loads, and the potential for substantial temporal variation in parasite assemblages on a host fish (Begg and Waldman 1999; Ferrer-Castelló *et al.* 2007).

Understanding the links between otolith chemistry and parasitism is a difficult task. In many cases it will be unclear whether differences in otolith chemistry result from differences in parasite loads, or whether differences in otolith chemistry and parasite loads are both products of the different geographic and environmental histories experienced by separate fish stocks or populations. It is only through our more detailed knowledge of the movement patterns of *T. novaezelandiae* within Jervis Bay, gained via a larger study that used otolith-chemistry signatures as natural population-specific tags and which identified each subpopulation or migratory contingent (E. C. Heagney, B. M. Gillanders and I. M. Suthers, unpubl. data), that we have been able to isolate the effect of *Ceratothoa* parasites on otolith chemistry within each regional or migratory group. Understanding and accurately interpreting the links between otolith chemistry and parasitism will require more complex sampling designs that incorporate otolith-chemistry signatures in longer-term tag–recapture experiments or use more conventional tag–recapture or acoustic and satellite tagging techniques to provide data on the movement or migration patterns experienced by each group sampled.

Our results have also highlighted the potential for parasitism to confound otolith-chemistry studies. Parasite assemblages and loads are not uniform within a population, so differential effects of parasitism may create differences in otolith chemistry within a single stock or subpopulation unit, similar to those observed for Li : Ca, Mg : Ca and Sr : Ca in our study, which may be incorrectly interpreted as fine-scale population structuring. Parasite assemblages and loads are also heterogenous among populations, so, depending on the magnitude and direction of effects of parasitism relative to regional or population-based differences in otolith chemistry, differential effects of parasitism may obscure population-based differences in otolith chemistry. Although our results are drawn from a relatively small sample size and relate to one specific parasite–host interaction, the ubiquitous distribution of fish parasites in the marine environment suggests that parasitism may be an important factor in a much broader range of otolith-chemistry studies. Further insights into the effects of parasitism may be key to understanding physiological effects on otolith chemistry, and assist in the accurate interpretation of otolith-chemistry data.

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