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REPORT

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Stable isotopes in fish otoliths discriminate between lagoonal and oceanic residents of Taiaro Atoll (Tuamotu Archipelago, French Polynesia)

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Abstract The fully enclosed Taiaro lagoon is hypersaline (42.5 psu) and non-tidal; constant salinity and water level result from strong evaporation balanced by low percolation through the lagoon floor. Seawater can flow over the atoll rim during exceptionally high seas and may then replenish lagoonal communities with propagules of oceanic origin. The distinctive water chemistry of the lagoon suggests a possible way of identifying these immigrants. We established this potential by analysing stable isotopes of carbon and oxygen in the recent growth layers of otoliths of two adult reef fishes, *Chaetodon ulietensis* and *Acanthurus triostegus*, collected from both sides of the atoll rim. Fish from the two locations were discriminated by their isotopic signatures, suggesting that analysis of the microchemical signatures deposited during the larval development could be used in future work to determine which individuals and species complete their life-cycles in this unusual lagoon.

Key words Coral reef fishes · Life cycle · Larval dispersal · Microchemistry · Spawning source

Introduction

Taiaro Atoll (15°45′S, 144°38′W) surrounds one of the most closed lagoons in French Polynesia and is disconnected from the ocean during normal conditions (Galzin et al., this volume). Despite its apparent isolation, the fish fauna of the lagoon of Taiaro is relatively rich (Galzin et al., this volume). Elsewhere in French Polynesia, it is normal for larval fish to travel through breaking surf to access lagoonal habitats (Dufour et al. 1996) and it is possible that some or all of these fish are introduced into the lagoon during rare episodes of oceanic ingress. Alternatively, some or all of these species may sustain local populations within the enclosed lagoon (Leis 1994; Leis et al., this volume). The 1994 Franco-Australian expedition to Taiaro sought to resolve this question, but was limited in its capacity to collect such evidence by direct sampling given the short duration of the visit to the atoll. Thus, our goal was to find proxies that might be used to determine the origin(s) of the reef fishes within the lagoon.

One way to discriminate between different fish stocks is to analyse natural tags that originate from the local environment or which reflect closed populations (Edmond et al., 1989; Planes et al. this volume). The hypersalinity of Taiaro Lagoon encouraged us to try microchemical analysis of fish otoliths. Stable isotopes in calcium carbonate have been used in various marine organisms as environmental recorders, especially fish (Devereux 1967; Mulcahy et al. 1979; Iacumin et al. 1992; Radtke et al. 1996). Although largely used as thermometers (Devereux 1967), O^{18} in calcium carbonate has also been used to record changes in salinity (Cole et al. 1993). Since Kalish (1991a) noted that stable isotopes of carbon and oxygen have great potential for understanding fish biology, there has been new interest in hindcasting a host of factors from the permanent records of otoliths (Kalish 1989, 1991b; Iacumin et al. 1992; Gillanders and Kingsford 1996; Radtke et al. 1996).

Because Taiaro Lagoon is persistently hypersaline due to high evaporation and low ingress of oceanic water (Chevalier and Salvat 1976), we predicted that this might be reflected in unique chemical fingerprints

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of otoliths from fish exposed to such conditions. Initially, we measured the salinity and temperature of the lagoon to estimate the hydrological balance and to determine whether stable isotopes of carbon and oxygen differed enough between lagoon and ocean waters for this method to be theoretically effective. Finally, we analysed the composition of otoliths from fish collected in both environments to test whether there was potential for these structures to identify the origin(s) of the fishes living inside Taiaro Atoll.

Methods

Hydrology

Hydrological measurements were made in the sea and the lagoon between 13–19 February 1994. Salinity and temperature were profiled with a CTD probe (MORS SLS 57) at baseline stations in the ocean located over the 200 m and 500 m isobaths. These parameters were also recorded from the ocean in 1992 (Rancher and Rougerie 1995). In the lagoon, similar data were collected on four different days from five stations along a transect (NE–SW) that was oriented with the wind (Fig. 1).

Simple instrumentation was used for recording water levels. Inside the lagoon, we recorded water level to the nearest centimetre on a graduated stick that was fixed inside a small porous pool to dampen wind waves. Outside the lagoon, the tidal signal was monitored by fixing the CTD at 20 m on the reef slope. While its pressure sensor was not highly sensitive, we did observe the passage of tides. We also measured the relative water levels of the lagoon and the ocean with a geometric level positioned in the lowest point of dry land separating the two water bodies. The best measurements were obtained during calm sea conditions on 15 and 17 February; four different readings were taken on each date.

Otoliths

We used otoliths from a subset of fish that were collected from the lagoon and the ocean for genetic analysis. Planes et al. (this volume) collected 50 *Chaetodon ulietensis*, a microcarnivorous butterflyfish, and 50 *Acanthurus triostegus*, an herbivorous surgeonfish, from each environment. All individuals were large (14-16 cm SL for *C. ulietensis*; 15–22 cm SL for *A. triostegus*) and at least three years of age (Planes et al., this volume). On capture, sagittae were removed from each fish and stored dry in glass vials. In the laboratory, otoliths from four individuals representing each combination of species and habitat (i.e. 16 fish in total) were cleaned ultrasonically in distilled water, then left in 12% sodium hypochlorite for 48 h to oxidise any organic matter adhering to the carbonate matrix. Each otolith was rinsed with distilled water and a small piece was broken from its margin consisting of material deposited after the colonisation of either lagoonal or oceanic habitat.

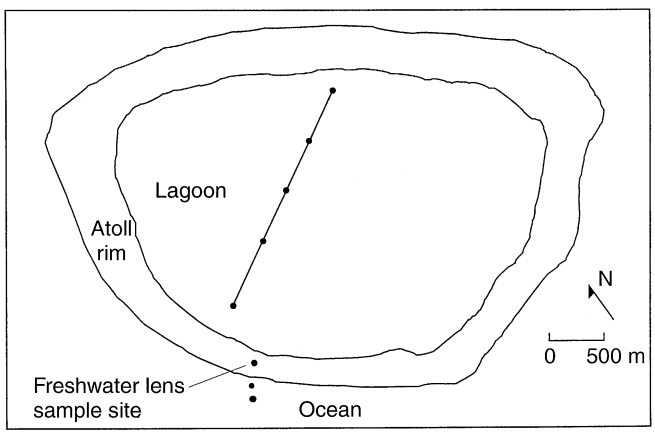

Fig. 1 Location of the hydrological stations for Taiaro Lagoon, groundwater, and ocean

Stable isotope analyses

Six water samples were collected for stable isotope measurements. Two stations were sampled at 5 and 20 m in the ocean; one station was sampled in the freshwater lens located 1 m under the surface of the atoll rim; three stations were sampled at 1 m, 10 m and 20 m in the lagoon (Fig. 1). At each station, one 20 ml glass bottle of water was taken for $\delta^{18}O$ measurements and one 125 ml glass bottle was taken for $\delta^{13}C$ measurements. Both samples were treated with 1 ml of a saturated $HgCl_2$ solution to prevent microbial growth.

Standard technical procedures were used for sample preparation: water-CO_2 equilibration at 25 °C for the $\delta^{18}O$ analyses of water (Epstein and Mayeda 1953) and extraction of the total inorganic carbon fraction for the analyses of aqueous CO_2 (Kroopnick 1971). The CO_2 was analysed with a triple collector mass spectrometer (Isogas-Sira 9). Analytical precision was 0.01 (2s) and reproducibility was 0.05‰. Otolith fragments were heated to 350 °C to remove all traces of organic matter, then ground with an agate mortar. About 100 µg of the resulting powder was reacted with phosphoric acid at 90 °C in an in-line system coupled with another mass spectrometer (Fisons-Optima). Analytical precision was 0.01 (2s); reproducibility was 0.01‰ for $\delta^{13}C$ and 0.03‰ for $\delta^{18}O$. Isotopic compositions were expressed in the conventional δ notation corresponding to the relative difference in ‰ of the isotopic ratios R_s and R_r of the sample and reference, respectively:

$$\delta(s) = ([R_s - R_r] - 1) \times 10^3 \quad \text{where } R = {}^{18}O/{}^{16}C \text{ or } {}^{13}C/{}^{12}C$$

The references were PDB (Peedee belemnite) for carbonate (Craig 1957) and SMOW (Standard Mean Ocean Water) for the seawater (Craig 1961). The equations of Grossman and Ku (1986)

$$\delta^{18}O = 4.70 - 0.228\ (T\,^{\circ}C) + \delta w$$

$$\delta^{13}C = 2.40 - 0.108\ (T\,^{\circ}C) + \delta CO_2$$

were used to predict the values of $\delta^{18}O$ and $\delta^{13}C$ expected in biogenic aragonite that has been precipitated in isotopic equilibrium with ambient levels of δw and δCO_2 for stable isotopes of oxygen and carbon respectively.

Data analysis

Each otolith was specified by two stable isotope ratios, and represented in a nearly orthogonal design (one otolith was lost). Consequently, the results were analysed by the STATISTICA as a "planned comparisons multivariate analysis of variance (MANOVA) with samples as the independent variable; isotopes as the dependent variables, and the missing value accommodated by case-wise deletion. The homogeneity of data within cells was tested by Box's M test. A discriminant function analysis was also done and 95% confidence limits for the adjusted means in canonical space were calculated by the formula: $(5.991/n)^{1/2}$. Post-hoc comparisons among treatment means in the raw data were done as Student-Newman-Keuls (SNK) tests.

Results

Hydrology

Figure 2 shows vertical profiles of temperature and salinity in the lagoon under typical conditions, with easterly trade winds of 8-10 m s^{-1}. Salinity was uniform through the water column and no gradients were detected in any of the daily transects. Lagoonal temperatures, which ranged between 30.4–29.7 °C, were higher by 0.5-1 °C than those of the ocean surface. During five days of observations, the lagoon level remained constant, despite external tidal amplitude of 20 ± 2 cm. Eight measurements taken over two days showed that the lagoon surface was approximately 30 cm below mean sea level.

Stable isotopes in the environment

The standardised abundance of stable isotopes of oxygen and carbon differed between water samples from Taiaro Lagoon and the ocean (Table 1), with relatively little internal variation in each water body. The uniformity of stable isotopes throughout the lagoonal water column shows that this enclosed water body is well mixed, consistent with the lack of thermal stratification (Fig. 2). The $\delta^{18}O$ values of lagoonal waters ($\sim 1.8‰$ SMOW) were enriched by almost 1‰ relative to ocean waters ($\sim 0.8‰$ SMOW). This enrichment associated with an increase in salinity of about 7‰ is clearly due to evaporation. The ratio of the isotopic and salinity enrichment (0.14) was close to the value (0.11) characteristic of equatorial oceanic waters (Craig and Gordon 1965). The $\delta^{18}O$ value ($-3.3‰$ SMOW) of the brackish water from the superficial aquifer was consistent with the values expected from local rainfall (Yurtsever 1975). The $\delta^{13}C$ values of CO_2 in the surface ocean waters around Taiaro (Table 1) were typical of oligotrophic waters in this part of the Pacific (Laube-Lenfant and Pierre 1994). Lower values in the lagoon are consistent with inputs of $\delta^{13}C$-depleted CO_2 from remineralization of organic matter. This is a well-known eutrophication effect in closed environments (Laube-Lenfant and Pierre 1994). The very low value of $\delta^{13}C$ in the freshwater lens indicates that the total inorganic carbon pool is contaminated by degradation products of organic matter from terrestrial biota. Thus, the three aqueous environments (ocean, lagoon and underground water) of Taiaro Atoll had distinctive values for stable isotopes of oxygen and carbon. These differences should be reflected during the precipitation of carbonates.

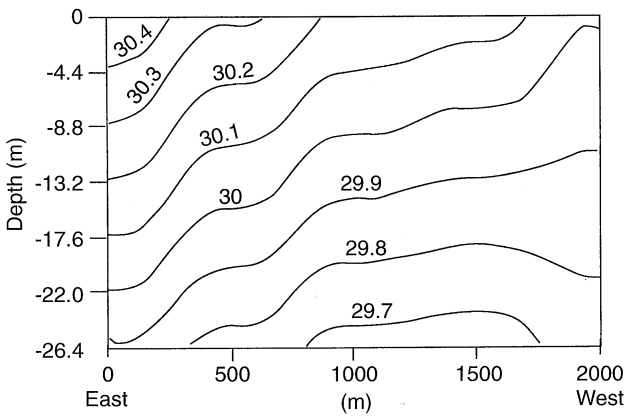

Fig. 2 Average isothermal structure along the lagoon transect (downwind from right to left)

Table 1 Values of $\delta^{18}O$ and $\delta^{13}C$ measured in waters around Taiaro Atoll relative to international standards: SMOW–Standard Mean Ocean Water, PDB–Peedee belemnite

Collection site	$\delta^{18}O$/SMOW	$\delta^{13}C$/PDB
Ocean (5 m)	+ 0.78	+ 1.37
Ocean (10 m)	+ 0.76	+ 1.38
Groundwater	− 3.27	− 13.02
Lagoon (surface)	+ 1.81	+ 0.25
Lagoon (10 m)	+ 1.84	+ 0.15
Lagoon (20 m)	+ 1.83	+ 0.18

Stable isotopes in fish otoliths

Figure 3 shows the values of stable isotopes recovered from otoliths in the four samples and compares these results against the values of isotopes expected in biogenic aragonite precipitated at 29 °C in isotopic equilibrium with ambient water (Grossman and Ku 1986). Higher temperatures, as found in the lagoon, shift these predicted values to lower $\delta^{18}O$ and higher $\delta^{13}C$. The slope of a regression fitted by least-squares to the total data set matched the theoretical predictions, given the differences between lagoon and ocean water (ignoring the small temperature difference), but the absolute levels of isotopes in the otoliths were not deposited in isotopic equilibrium with the ambient waters. In addition, the two species showed consistently different degrees of disequilibrium (Fig. 3).

All treatments displayed individual variability in the estimates, especially *C. ulietensis* from the lagoon (Fig. 3), though Box's M test did not detect significant heteroscedascity ($P = 0.48$). Nonetheless, the MANOVA detected highly significant differences among the samples (Pillai-Barlett Trace, $P = 0.002$) rejecting the overall null hypothesis for both planned comparisons (oxygen, $P < 0.001$; carbon, $P = 0.027$). Fig. 3 shows minimal overlap of 95% confidence limits for O^{18} among any of the samples and this was reflected in the post-hoc test results: both SNK (sources within species), $P < 0.001$; both SNK (species within sources), $P < 0.005$. In contrast, the confidence limits for $\delta^{13}C$ showed considerable overlap among most

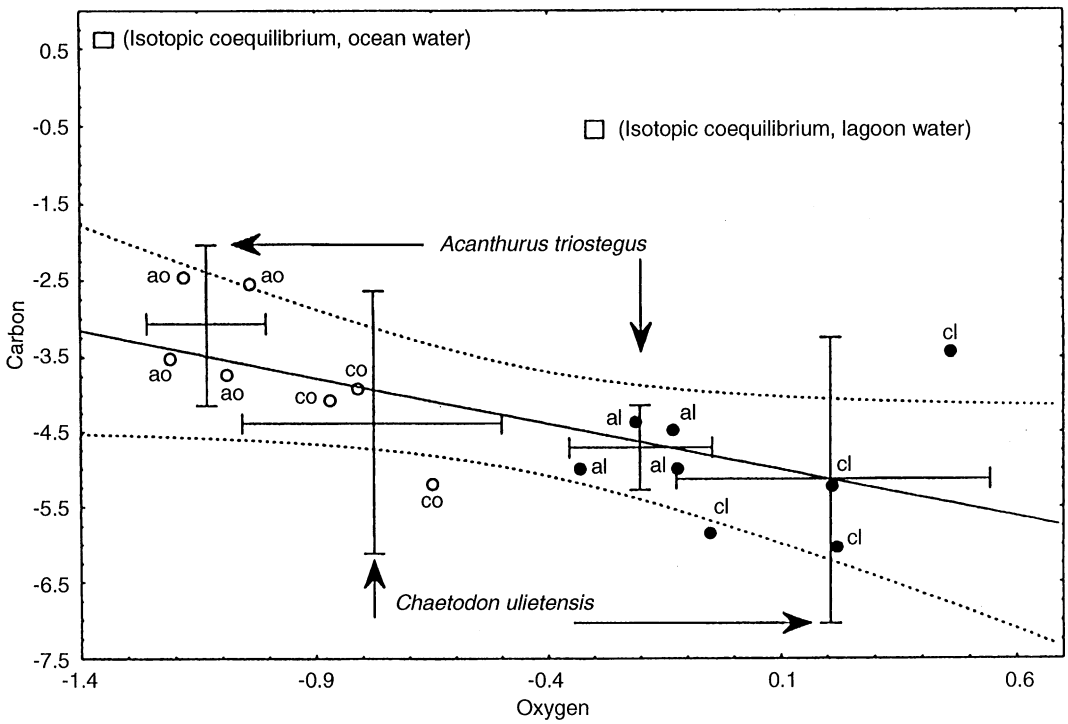

Fig. 3 Isotopic ratios of $\delta^{18}O$ and $\delta^{13}C$ in the otoliths of *Chaetodon ulietensis* and *Acanthurus triostegus* collected from Taiaro Lagoon (*filled circles*, species identified by *cl* and *al*) and the ocean (*open circles*, species identified by *co* and *ao*). Mean values ($\pm$ 95% CL) are shown for each sample group along with a regression ($\pm$ 99% CL) fitted to all data by "least-squares". Values expected in biogenic aragonite precipitated in isotopic equilibrium at 29 °C provide a reference point for each habitat

samples, though less so for *A. triostegus* (Fig. 3), resulting in no difference between samples of *C. ulietensis* (SNK, $P = 0.43$), probable difference between samples of *A. triostegus* (SNK, $P = 0.04$), and a difference between species only in the ocean (SNK, $P = 0.04$; lagoon, $P = 0.48$). Inspection of the detrended residuals suggested that at least one of the estimates for carbon may be an outlier and thus the test results may be conservative, especially given the small and unbalanced samples.

Despite the imprecise result from carbon, the first axis of a discriminant function analysis, which is a combination of both isotopes given their covariance (Fig. 3), explained $> 99\%$ of the total variation among observations (Fig. 4). Such an outcome indicates congruence (i.e. lack of interaction) between the two variables and also shows (from their relative separations in canonical space) that the combined signal was equally powerful at discriminating between sources for species of different trophic status, despite the fact that their absolute values were not the same (Fig. 4).

Discussion

Hydrology

Taiaro Lagoon showed heating effects, with slightly warmer water at the surface, but no stratification (Fig. 2). Slanted isotherms, consistent with wind-induced circulation, suggest that warm surface water was moving downwind resulting in some depression of the isotherms on the western side of the lagoon. To balance this movement within an enclosed system, a reverse circulation can be anticipated along the bottom with a weak upwelling on the opposite side of the lagoon. Such internal circulation agrees with circulation models described from other less-enclosed atolls (Von Arx 1948; Lenhardt 1991) and the distribution of passive zooplankton sampled during the 1994 expedition (Carleton and Doherty, this volume). The average lagoon salinity was uniform at 42.6 $\pm$ 0.2 psu, indicating well-mixed conditions.

Our observations match those recorded during an earlier visit in 1972 (Chevalier and Salvat 1976), which found that the lagoon was about 6‰ saltier and 1 °C warmer than the ocean. In this part of the Pacific, evaporation exceeds precipitation ($E - P = 0.8$ m y^{-1}, Rancher unpublished data). Therefore, for salinity in Taiaro Lagoon not to increase indefinitely, we must assume that salt is exported back to the ocean by percolation through the reef matrix. Similarly, the persistence of water in the lagoon, at just a few decimeters under sea level, implies recharge, either from episodic

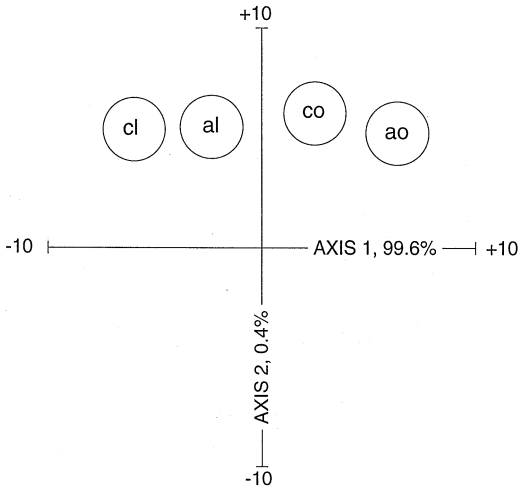

Fig. 4 Sample means (surrounded by 95% CL) on the first two discriminant functions with the portion of total variance explained by each function. Symbols as in Fig. 3

overtopping of the land barriers or continuous seepage through the reef matrix. The fact that we recorded no tidal influence in the lagoon during our visit suggests that permeability is low. Thus, we assume that water exchange is dependent on natural convection cells driven by density gradients in temperature and salinity (Rougerie and Wauthy 1993).

Stable isotopes in fish otoliths

Previous studies on stable isotopes in otoliths have been directed at understanding the physiology and/or ecology of fishes, especially ontogenetic changes in metabolism or habitat (Mulcahy et al. 1979; Kalish 1991b; Gillanders and Kingsford 1996). Kalish (1991a) and Iacumin et al. (1992) have recently compared oxygen and carbon isotope fractionations in fish otoliths from a wide range of species. Both reviews concluded that $\delta^{18}O$ values in otoliths depart little from isotopic equilibrium (but see Radtke et al. 1996) while $\delta^{13}C$ values are often lower than expected due to the incorporation of metabolically-derived CO_2. Our study provides excellent agreement with these general conclusions and has the advantage of precise calibration of fractionation for both isotopes because we measured the ambient parameters (temperature, $\delta^{18}O$ of water, $\delta^{13}C$ of dissolved CO_2), which are unlikely to deviate widely under the oligotrophic conditions of the eastern Pacific Ocean.

For $\delta^{13}C$, we detected strong, albeit consistent, departure from equilibrium, suggesting that carbon incorporation in otolith carbonate from the aqueous CO_2 reservoir is controlled by biological processes. Kalish (1989, 1991a) noted that carbon isotope disequilibrium in otoliths seems to be related to metabolic rate, which could be expected to change among species; perhaps also among individuals and during ontogeny. The variation that we observed among individual *C. ulietensis* may be due to such effects (Fig. 3). Despite this variation, the analysis detected consistent differences for both factors (species and habitat) in the experimental design. Considered separately, oxygen performed better than carbon at distinguishing among our samples (species and sources), but we detected no interaction between the two isotopes in our analyses. Since carbon did separate some samples in the post-hoc comparisons, we assume that it was not a redundant variable and the discriminant function analysis showed that the combination of both signals explained $>99\%$ of the variation in the total data set. Consequently, we suggest that the two isotopes offer the best chance of discriminating the ambient conditions experienced by fish and advocate that isotopic analysis of material near the primordium (deposited during pelagic development) can be used in future research to identify the source(s) of fish found within the enclosed lagoon of Taiaro Atoll using modern techniques of microchemical analysis (Smalley et al. 1989; Sharp 1992; Radtke et al. 1996).

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