

Testing larval fish dispersal hypotheses using maximum likelihood analysis of otolith chemistry data

Stuart A. Sandin^{A,D}, James Regetz^B and Scott L. Hamilton^C

^ACenter for Marine Biodiversity and Conservation, Scripps Institution of Oceanography, La Jolla, CA 92093-0202, USA.

^BDepartment of Ecology and Evolutionary Biology, Princeton University, Princeton, NJ 08544-1003, USA.

^CDepartment of Ecology, Evolution, and Marine Biology, University of California, Santa Barbara, CA 93106, USA.

^DCorresponding author. Email: ssandin@ucsd.edu

Abstract. Otolith chemical analyses enable researchers to follow the dispersal pathways of individual fish through time. Given that water masses have spatially or temporally variable chemical signatures (or correlates thereof) and that this variability can be modelled statistically, we have the potential to describe a fish's dispersal history by examining a temporal transect of elemental concentrations throughout the otolith generated from laser ablation inductively coupled plasma mass spectrometry. Statistical analyses tend to focus on temporal trajectories of individual elements or analyse multiple elements at single points in time. We have developed a customised statistical technique allowing detailed exploration of elemental signatures using maximum likelihood methods. The benefit of this approach is the ability to model chronological series of otolith measurements for all sampled fish and all elements simultaneously, while providing explicit treatment of variability in the data. We used data from a Caribbean fish population to compare traditional analysis techniques with this likelihood-based approach, showing their relative capacities to test among alternative hypotheses regarding the dispersal trajectories of individual fish. By incorporating information specific to the species' natural history and to the analytical techniques, we can explore more detailed models of fish movement than were possible using pre-existing approaches.

Extra keywords: customised estimator, data-driven statistics, larval dispersal and retention.

Introduction

The information recorded within the otoliths of bony fishes can be used to consider a growing suite of questions regarding the pre-capture movements and behaviours of individuals. In many cases, the chemical composition of the layers of otolith material reflects characteristics of the water through which an individual fish has passed (Campana 1999; Bath *et al.* 2000). The effective use of otolith chemical techniques for resolving the movements of fish requires three conditions to be satisfied. (1) Geographic characteristics must be recorded in the otolith. In particular, the otolith material accreted during a period of time must reflect a spatially and/or temporally variable chemical signature (e.g. caused by variation of water chemistry, temperature, salinity; reviewed by Campana 1999). (2) Chemical analysis techniques must offer sufficient precision to resolve chemical signal variability in the otolith resulting from differences of chemical uptake across space and/or through time (Campana *et al.* 1994; Fowler *et al.* 1995). (3) Statistical techniques must be robust enough to test specific biological questions accurately by accounting for

the noisy, highly dimensional data characteristic of chemical analyses.

In natural populations, otolith elemental signatures have been used to identify different stocks (Edmonds *et al.* 1991; Campana *et al.* 1999, 2000), determine spawning grounds (Campana *et al.* 1994), distinguish juvenile nursery areas (Thorrold *et al.* 1997, 1998b; Gillanders and Kingsford 2000; Forrester and Swearer 2002), and identify natal rivers (Thorrold *et al.* 1998a, 2001) and migratory patterns (Arai *et al.* 2003) of anadromous fishes. Different statistical approaches are necessary for examining the questions and assumptions posed by the broad applications of otolith chemistry. Clustering techniques can be used to group fish based upon similarities in concentrations of multiple elements in the whole otolith (i.e. solution-based approaches), multiple elements at a single point in time (i.e. analysis of otolith cores), or temporal trajectories of a single element across the otolith. Individual fish are placed into groups based on common histories, such as dispersal pathways (Swearer *et al.* 1999) or coherent fish stocks (Campana *et al.* 1999). Using a priori

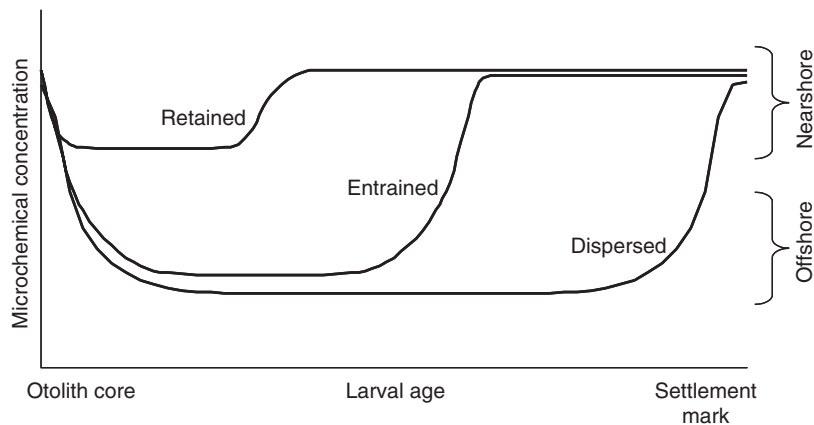


Fig. 1. Hypothesised chemical profile under three larval movement trajectories. Retained fish move only a short distance offshore, remaining within water with a nearshore chemical signature. Entrained fish enter the offshore waters early in their pelagic larval duration (PLD), but return (either by passive or active processes) to the nearshore waters for the latter part of their PLD. Dispersed fish spend almost all of their PLD in offshore waters. Note that the distinction between entrained and dispersed fish is only the amount of time spent in nearshore waters before settlement. Redrawn from Swearer (2000).

groupings (i.e. sites of collection) many researchers have used discriminant function analysis to successfully discriminate among populations of fish based on otolith elemental signatures and accurately classify fish to their site of origin (Campana *et al.* 1994; Rooker *et al.* 2001; Swearer *et al.* 2003). By considering the distributions of elemental signatures in mixed stocks, maximum likelihood-based classification approaches (derived from the work of Millar 1987) can offer more accurate population-level estimates of connectivity among stocks (Campana *et al.* 1999; Thorrold *et al.* 2001; Gillanders 2002). However, these classification techniques are limited to comparing differences in multiple elements across the whole otolith (Rooker *et al.* 2001; Swearer *et al.* 2003) or multiple elements at one point in time (often the otolith core; Campana *et al.* 1994; Thorrold *et al.* 2001; Gillanders 2002). By examining elemental profiles through time, it has been possible to resolve differences in migratory or dispersal histories among individuals within a population (e.g. Swearer 2000; Arai *et al.* 2003). Largely, such studies rely upon profiles of only a single element among members of the population to assign each individual to a migratory or dispersal pattern.

As the field of otolith chemical analysis matures, increasingly detailed questions can be asked with the technique (Campana and Thorrold 2001). Increases in the accuracy and precision of analytical techniques are facilitating this maturation. Additionally, the growing background understanding of elemental incorporation into otoliths, including controlled validation studies (e.g. Mugiya *et al.* 1991; Geffen *et al.* 1998; Bath *et al.* 2000; Elsdon and Gillanders 2003) and the characterisation of chemical variation across space (Thorrold *et al.* 1998a; Forrester and Swearer 2002; Gillanders and Kingsford

2003), allows more focused questions to be addressed. By clearly stating the assumptions of each analytical approach and by validating the legitimacy of these assumptions, the use of otolith chemistry will be greatly expanded to tackle the next generation of biological questions. Such advances depend on a tailored set of statistical approaches that account for increasingly complex methodology and system-specific details.

Here we present a technique for resolving fine-scale larval dispersal patterns based on existing knowledge of natural history and elemental deposition in the otolith. Focusing on a population of coral reef fish *Thalassoma bifasciatum* around St Croix, United States Virgin Islands, we attempt to describe the variability of larval dispersal trajectories across individuals. The natural history of *T. bifasciatum* and the nearshore oceanography of St Croix (summarised well in Swearer 2000 and Harlan *et al.* 2002) suggests that three distinct pathways may characterise the range of pre-settlement movement of larvae: (i) retention, in which larvae remain in nearshore waters until recruitment to the reef; (ii) dispersal, in which fish spend the most of their larval period in oceanic waters (called 'dispersal' because of the supposed importance of a long pelagic period to allow for successful transit among islands); and (iii) entrainment, in which larvae from oceanic waters are entrained in a nearshore water mass for a substantial period before recruiting to the reef (Fig. 1). Previous attempts to identify the movement trajectories of individual fish have used the chronological series of only a single element (e.g. Pb, Swearer 2000; Sr, Arai *et al.* 2003). However, multiple elements are often collected simultaneously with laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS), and statistical power to discriminate among

different dispersal histories might be lost in treating each element separately. Using maximum likelihood methodology, we provide a data-driven approach to best classify individual fish into one of these three movement trajectories, simultaneously using the temporal profiles of multiple elements and accounting for process uncertainty and machine error. For comparative purposes we present results of more traditional statistical analyses and note differences in the assumptions and capabilities of each approach.

Materials and methods

Fish collections

Newly recruited *T. bifasciatum* were collected from pavement reefs at Butler Bay and Jacks Bay during June, July, and August 2001. Recruit collections were timed to coincide with the broad recruitment pulse that occurs during the 2 weeks surrounding the new moon of each month (Caselle and Warner 1996). During each recruitment pulse, new recruits were captured by divers using hand nets and frozen at -80°C . Fish were collected at two locations on St Croix, Butler Bay on the eastern (leeward) coast and Jacks Bay on the western (windward) coast. Of these recruits, 200 fish (roughly equally distributed among two sites and 3 months) were selected randomly for otolith analyses.

Otolith elemental analysis

Otoliths were dissected from thawed fish and cleaned of adhering tissue. One sagitta from each fish was mounted sulcal side up on plastic slides using low viscosity epoxy resin (Epo-Thin epoxy resin, Buehler Ltd, Lake Bluff, IL, USA). Sagittae were polished to within $5\text{--}25\ \mu\text{m}$ of the core using a lapping wheel and $9\ \mu\text{m}$ and $3\ \mu\text{m}$ 3M diamond polishing film (South Bay Technology, San Clemente, CA) to expose inner growth layers. To remove contaminants and proteins from the otolith surface before analysis, sagittae were rinsed in ultra pure water (N-pure, resistivity $>18.1\ \text{M}\Omega$), soaked in semiconductor grade $15\% \text{H}_2\text{O}_2$ buffered with Suprapur $0.05\ \text{M}$ NaOH in acid-leached plastic trays for 1 h, rinsed again in N-pure, soaked and sonicated three times in N-pure for 5 min, rinsed a final time with N-pure and air-dried in a class-100 flow bench. Cleaning experiments indicated that soaking otoliths in H_2O_2 resulted in lower elemental concentrations and reduced variability in chemical signatures across the otolith transect compared with paired otoliths that had only been rinsed in N-pure (S. E. Swearer *et al.*, unpublished data).

We used a Finnegan MAT Element 2 sector field inductively-coupled plasma mass-spectrometer (ICPMS) and a GV ultraviolet-microprobe 266 nm Nd:YAG 266 nm laser ablation system for chemical analysis (GV Instruments Inc., Hudson, NH). The laser ablation system was outfitted with a helium aerosol carrier gas system in order to increase sensitivity through enhanced production and transfer of ablated particles to the ICPMS (Swearer 2000). Otoliths were placed in the sample cell and the core was located using a $400\times$ objective and a video imaging system (see Swearer 2000 for more details). We ablated and analysed the composition of individual pits along a transect stretching from the core to edge of the otolith (along the longest axis). Each pit consisted of eight laser pulses of $0.1\ \text{mJ}$ at $3\ \text{Hz}$, and ablated a volume $\sim 30\ \mu\text{m}$ in diameter and $\sim 10\ \mu\text{m}$ deep. Before acquiring data, we pre-ablated using two laser pulses to remove any surface contamination.

In each sample pit, we collected counts for the isotopes ^{24}Mg , ^{48}Ca , ^{55}Mn , ^{88}Sr , ^{138}Ba and ^{208}Pb . Molar ratios of analyte to Ca were calculated using the ratio of each isotope to ^{48}Ca and an elemental mass bias correction calculated from calibration standards (repeated after every three to five otoliths) with known analyte to Ca ratios. Blanks were taken at the beginning of each sequence and background counts of each element were subtracted from those detected by the ICPMS. We analysed

solid glass standard reference material (National Institute of Standards and Technology reference material 612) along with the samples to maintain instrument analytical precision; estimates of precision (as % relative standard deviation) were 4.8% for Mg:Ca, 4.7% for Mn:Ca, 8.4% for Sr:Ca, 4.6% for Ba:Ca, and 6.7% for Pb:Ca. Limits of detection (LOD) were calculated as 3 s.d. of blanks; LOD ratioed to mean Ca intensities yielded Me:Ca detection limits of $0.027\ \text{mmol mol}^{-1}$ for Mg:Ca, $0.065\ \mu\text{mol mol}^{-1}$ for Mn:Ca, $0.053\ \text{mmol mol}^{-1}$ for Sr:Ca, $0.11\ \mu\text{mol mol}^{-1}$ for Ba:Ca and $0.034\ \mu\text{mol mol}^{-1}$ for Pb:Ca. Values below the detection limits of the instrument and occasional negative values (as a result of background subtraction error) were included in the statistical analyses and explicitly modelled as machine error in the maximum likelihood algorithm.

For each fish, five to eight pits were ablated between the core and the settlement mark. The daily rings in the otoliths were counted using a compound microscope and image analysis system (Image Pro 4.5, Media Cybernetics Inc., Silver Spring, MD) before and after laser ablation and each ablation pit was assigned an age. The age of the fish at each pit was used in the explicit likelihood model presented here. However for the traditional statistics, each pit was categorised into one of five developmental periods (i.e. beginning, early, middle, late, and end of the larval phase). The length of each developmental period was determined by dividing the pelagic larval duration (PLD) of each individual by five. If multiple pits fell within one developmental period, the concentrations of each element were averaged. Pits from the otolith core and those that overlapped with the settlement mark were excluded to ensure that all elemental concentrations were from larval development only.

We filtered the data in a post-processing step to prevent potentially contaminated pits and concentration readings from biasing the likelihood model. We removed elemental concentration values outside of reasonable threshold concentrations. Maximum thresholds were set based on expert opinion as follows: Mg:Ca, $1.5\ \text{mmol mol}^{-1}$; Mn:Ca, $10.0\ \mu\text{mol mol}^{-1}$; Ba:Ca, $5.0\ \mu\text{mol mol}^{-1}$; and Pb:Ca, $1.5\ \mu\text{mol mol}^{-1}$. These correspond to values approximately an order of magnitude greater than the average values recorded. Note that the use of alternative, reasonable threshold values did not change significantly the groupings estimated by any of the following statistics. Finally, because Sr:Ca values varied little, no data removal was required for this element.

Models: likelihood procedure

All likelihood inference was based on a simple but biologically reasonable model of larval dispersal. The first main assumption is that there are two water masses with distinct chemical signatures: nearshore and offshore. The second structural assumption is that each fish is characterised by one of three different dispersal syndromes. Retained individuals are those that reside in the nearshore water mass for the full larval duration. Dispersed individuals are those that reside in the offshore water mass until recruitment to the benthic habitat. Entrained individuals are those that initially reside in the offshore water mass, but move nearshore before settlement (Fig. 1). Note that we omit data from the otolith core and from the settlement mark, and therefore are only modelling the 'pelagic' period of the larval movement history.

Given this model, each entrained individual should exhibit a coherent shift in chemical signature somewhere along the series of otolith pits corresponding to the time at which it moved into the nearshore environment. We refer to the first pit measurement taken after this transition as the switch point. For a fish with n pits, the empirical support for each of the $n - 1$ switch points was evaluated using a simple least-squares criterion. Specifically, for switch point k , pre-switch means were calculated for each element over the first $k - 1$ pits, and post switch means were calculated for each element over the last $n - k + 1$ pits. The squared deviations from these means were then summed over all elements and all pits. The switch point associated with the smallest summed squared deviation was regarded as the most likely switch point. Note that this is

equivalent to the maximum likelihood switch point under the assumption that elemental concentrations are normally distributed before and after the switch point.

If the true dispersal syndrome of each fish were known, it would be possible to determine the distribution of each element within each of the water masses. The putative nearshore elemental means and variances of elemental concentrations could be calculated using all pits from retained fish and post switch pits from entrained fish. Similarly, the putative offshore means and variances of elemental concentrations could be calculated using all pits from dispersed fish and pre-switch pits from entrained fish. These calculations follow from our basic assumptions about the relationship between each dispersal syndrome and the associated water masses (Fig. 1). Corresponding with the distributions of our data, our model assumes that the elemental concentrations are normally distributed within each water mass, but this assumption could be easily relaxed for cases with non-normal distributions.

Although we do not know the true dispersal syndromes, we designed a stochastic optimisation algorithm to determine the most likely assignments for all fish. To begin this procedure, each fish was randomly assigned a dispersal syndrome, and means (μ_N , μ_O) and variances (σ_N^2 , σ_O^2) of elemental concentrations from nearshore and offshore waters, respectively, were calculated as described above. Each iteration of the routine proceeded as follows. A fish was randomly selected and the nearshore and offshore means and variances were recalculated without the pit measurements for this fish. The log-likelihoods associated with each of the three dispersal syndromes – retained ($\ln(L_R)$), entrained ($\ln(L_E)$), and dispersed ($\ln(L_D)$) – for one element were then calculated for this fish as follows:

$$\ln(L_R) = -\frac{n}{2} \ln(2\pi\sigma_N^2) - \frac{\sum_{t=1}^n (\mu_N - x_t)^2}{2\sigma_N^2} \quad (1a)$$

$$\ln(L_E) = -\frac{k-1}{2} \ln(2\pi\sigma_O^2) - \frac{\sum_{t=1}^{k-1} (\mu_O - x_t)^2}{2\sigma_O^2} - \frac{n-k+1}{2} \ln(2\pi\sigma_N^2) - \frac{\sum_{t=k}^n (\mu_N - x_t)^2}{2\sigma_N^2} \quad (1b)$$

$$\ln(L_D) = -\frac{n}{2} \ln(2\pi\sigma_O^2) - \frac{\sum_{t=1}^n (\mu_O - x_t)^2}{2\sigma_O^2} \quad (1c)$$

where x_t is the concentration of an element at pit t . Note that for each datum (i.e. each elemental measurement from each pit), the log-likelihood is simply the logarithm of the Gaussian probability of obtaining that datum given the appropriate means and variances estimated over all other fish. The log-likelihoods associated with each syndrome were computed separately for each element, and summed over all elements. The overall likelihood that the selected fish was retained (R) is the summed log-likelihood over all of its pit-data based on the nearshore distributions; the likelihood that the selected fish was dispersed (D) is the summed log-likelihood over all of its pit-data based on the offshore distributions; and the likelihood that this fish was entrained (E) is the summed log-likelihood over all of its pre-switch point pit-data based on the offshore distributions plus the summed log-likelihood over its post switch point pit-data based on the nearshore distributions.

The dispersal assignment was then updated using a decision rule similar to that used by simulated annealing algorithms (Kirkpatrick *et al.* 1983), whereby ‘worse’ assignments are occasionally chosen as a way of escaping local optima. If the fish was initially assigned to one syndrome but one of the other two syndromes was determined to be the most likely, then the fish was always reassigned to the most likely syndrome. However, if the initial assignment was most likely, then the

fish was randomly reassigned to the next best syndrome with probability related to the difference in their log-likelihoods; otherwise it retained its initial assignment. An example will help to illustrate. If the fish was initially labelled R, and the most likely syndrome was determined to be E, then the fish was always reassigned to E. If the most likely syndrome was the initial assignment R, with E as the next most likely syndrome, then the fish was reassigned to E with probability L_E/L_R and kept as R otherwise; note that as R becomes increasing more likely than E, the probability of accepting a switch to E decreases. After applying this decision rule, the nearshore and offshore means and variances for each element were appropriately recalculated over all fish. This process was iterated sufficiently to assure that convergence on the most likely classification was achieved. The algorithm was repeated several times with different initial assignments and different random number seeds to ensure convergence towards a single solution.

We applied this three-syndrome model using the profiles of all five elements simultaneously, as well as all permutations of a model incorporating four out of the possible five elements. We deleted single elements to examine the influence of each element on the concordance of retained/entrained/dispersed (RED) assignments. It is important to note that the flexibility of the maximum likelihood approach allows an investigator to separate fish into any number of dispersal groupings, for any number of collected elemental profiles, and following any specified assumptions. (Readers who wish to view the original C++ code used for implementing the stochastic optimisation algorithm are encouraged to contact the authors directly.)

Models: comparative statistics

Repeated-measures analysis of variance (RM-ANOVA) was used to describe average changes in the concentration of each element with increasing fish age. The repeated effects were modelled using differences in concentration between the earliest age and each later age, and month of capture was included as an between-subjects factor. Note that the sphericity assumption of RM-ANOVA requires that differences in concentration share a common variance across all age comparisons (Huynh and Feldt 1970). Using Mauchly's criterion we found that data from all elements violated this assumption. To correct for this, we applied the conservative Greenhouse-Geisser adjustment to reduce the degrees of freedom used in each F-test. Following recommendation by Looney and Stanley (1989) we complemented the univariate analysis with analogous multivariate (MANOVA) tests, which do not rely on the sphericity assumption (O'Brien and Kaiser 1985).

In order to judge the relative performance of our likelihood model, two alternative statistical techniques for classifying fish into the RED categories were applied using these same data. First, using mean concentrations of each element across all pits for an individual, a three-means cluster analysis was used. Linking each of the three clusters to one of the RED classifications depended on the assumption that elemental concentrations tend to decrease with increasing distance from land (Bruland 1983). Therefore, the cluster with the highest concentrations of most elements was deemed ‘retained’, the cluster with the lowest concentrations was ‘dispersed’, and the intermediate cluster was ‘entrained’. Cluster analyses were performed using all elements, as well as using the five subsets of four element combinations, as in the likelihood analysis.

Second, a Tucker 3-way principal component analysis (PCA) was used to classify fish into RED categories (*sensu* Gemperline *et al.* 2002). This 3-dimensional principal component analysis (i.e. analogous to a PCA through time and across individuals) treats the multidimensionality of the data similarly to the likelihood model, and uses the concentrations of multiple elements within each individual pit to calculate PCA scores across the otolith transect. The Tucker 3-way PCA can account for consistent ontogenetic changes of elemental concentrations through the larval period and individual-specific differences in elemental deposition (i.e. as a result of residence in water masses differing in

Table 1. Outcomes of repeated-measures analysis for each sampled isotope

Data were separated into five age classes, evenly representing the pelagic larval duration of the individual fish. The age effect is based on within-otolith differences in isotope measurements between the first age class and each subsequent age class, whereas age \times month effect represents the effect of month of fish capture on the concentration change with age. Results are given for three different tests: a, unadjusted univariate F -test (note that the raw degrees of freedom vary across elements because of different frequencies of inductively coupled plasma mass spectrometry measurement errors); b, F -test based on Greenhouse-Geisser adjustment; c, Pillai's trace, a standard MANOVA test. Significant probability values are indicated by bold type

Element	Effect	Test	Value	F	d.f.	Probability
^{24}Mg	Age	a	1	23.19	4, 772	<0.0001
		b	0.630	23.19	2.5, 486.0	<0.0001
	Age \times month	a	1	0.55	8, 772	0.8175
		b	0.630	0.55	5.0, 486.0	0.7382
^{55}Mn	Age	a	1	38.13	4, 688	<0.0001
		b	0.911	38.13	3.6, 626.5	<0.0001
	Age \times month	a	1	2.89	8, 688	0.0036
		b	0.911	2.89	7.3, 626.5	0.0050
^{88}Sr	Age	a	1	115.2	4, 772	<0.0001
		b	0.765	115.2	3.1, 591.2	<0.0001
	Age \times month	a	1	9.66	8, 772	<0.0001
		b	0.766	9.66	6.1, 591.2	<0.0001
^{138}Ba	Age	a	1	25.50	4, 748	<0.0001
		b	0.510	25.50	2.0, 381.7	<0.0001
	Age \times month	a	1	1.94	8, 748	0.0517
		b	0.510	1.94	4.1, 381.7	0.1020
^{208}Pb	Age	a	1	7.24	4, 728	<0.0001
		b	0.769	7.24	3.1, 559.8	<0.0001
	Age \times month	a	1	0.56	8, 728	0.8116
		b	0.769	0.56	6.2, 559.8	0.7671
		c	0.023	0.53	8, 360	0.8315

trace element chemistry or physiological differences). The data were again lumped into five age categories and normalised to a common mean (1.0 for all elements). Based on the elemental axis from the first principal triad (as per Gemperline *et al.* 2002), a five-point plot (i.e. temporal trajectory of PCA scores for each age category) was made of the elemental concentrations for each fish. Using these plots, if the mean PCA score of the first two age categories was lower than the mean PCA score of the last two age categories, the fish was deemed 'entrained'. This categorisation was based on the expectation that large differences in PCA scores between the 'early' and 'late' age categories were indicative of a change in elemental concentrations across the otolith and likely signified larval residence in different water masses. This assumption is analogous to the 'switch point' concept used in the likelihood model to classify fish into the 'entrained' dispersal history. Of the remaining fish, if the mean PCA score of all age categories was above or below the overall mean (i.e. across data for all fish in all five age categories), the fish was labelled as 'retained' or as 'dispersed' respectively.

Concordance analysis

The concordance of classifications from the three grouping approaches (likelihood, cluster, and Tucker analyses) was compared using χ^2 analyses. Expected number of concordantly grouped fish between two analyses for each R, E, and D was calculated as the product of the proportions of R, E, or D, respectively, in each of the two analyses. The sum of the products for each R, E, and D was the expected proportion

of concordantly grouped fish. To account for the repeated comparisons among models, a threshold for concordance ($P < 0.001$) was used to conclude statistical significance.

Results

The RM-ANOVA analysis revealed significant differences in concentrations across age classes for all five elements (Table 1, Fig. 2). The Sr concentration tended to decrease with increasing age, while Ba showed higher values near the core and near the settlement mark of the otolith relative to the intermediate ages (Fig. 2a,b). For Pb, Mg, and Mn, the mean concentrations tended to increase with increasing fish age (Fig. 2c-e). The pattern of mean concentration change across fish age was affected significantly by month of fish capture for Sr and Mn (Table 1). Outcomes of the multivariate tests were identical to those of the univariate tests for Mg, Mn, Sr, and Pb but yielded a significant month effect for Ba. In Table 1 we only report results of Pillai's trace test, which appears to be the most robust to violations of assumptions concerning homogeneity of the covariance matrix (Olson 1976); however, Wilk's lambda, Hotelling-Lawley trace, and Roy's maximum root all yielded identical outcomes.

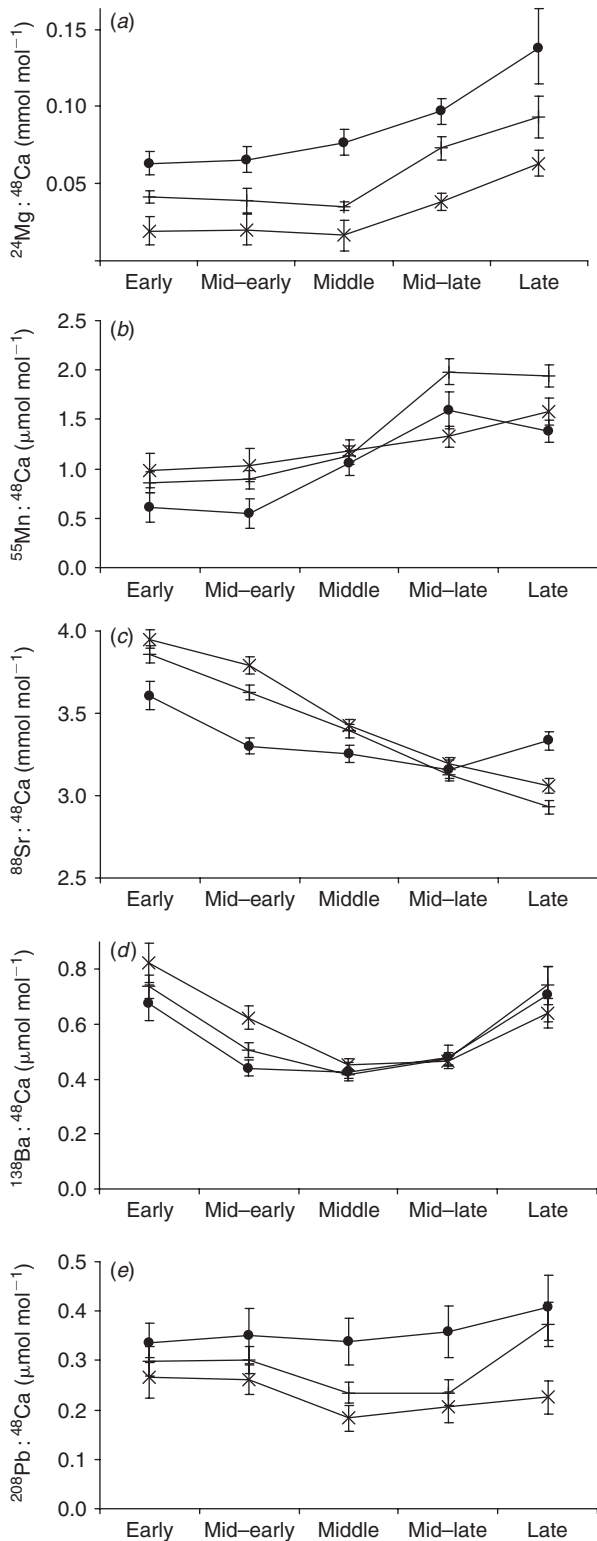


Fig. 2. Mean (with standard error bars) chemical concentrations across five fish age classes for each month of fish collection. (a–e) Standardised concentrations for ^{24}Mg , ^{55}Mn , ^{88}Sr , ^{138}Ba , and ^{208}Pb respectively. Mean concentrations change as a result of the combined effects of ontogenetic shifts of uptake rates and the average movements of fish in the sampled population. (●), June; (+), July; (×), August.

Using data from all five elements, the likelihood, cluster, and Tucker 3-way PCA analyses classified fish in R, E, and D groups more similarly than expected, with over 50% of fish identically classified between each pair of models (χ^2 test, $P < 0.001$ for each of three comparisons, Table 2). This high concordance occurred despite the difference of assumptions underlying each statistical approach. By repeating the likelihood and cluster analyses on subsets of four elements, the individual contribution of each element was explored. With the likelihood model, removal of Mn caused fish classifications to be altered significantly from the five-element model (Table 2). Models omitting any of the other four elements still resulted in over 90% concordance with the five-element model (Table 2). The importance of Mn to the likelihood model was reflected by the large difference of estimated Mn concentration between ‘nearshore’ and ‘offshore’ waters, with negligible differences for the other elements (Table 3).

Although Sr and Mg did not appear to be particularly informative elements with respect to our ability to discriminate dispersal syndromes in the likelihood model, including them in the analysis did not bias the results provided that there are at least some elements (e.g. Mn) that vary between nearshore and offshore water masses. In the absence of definitive a priori knowledge about which elements are informative, we argue that it is best to include all sampled elements in the analysis. If some elements, such as Sr and Mg in this case, prove to be uninformative, they will simply have little influence the results. However, if they provide at least a weak signal of water mass differences, this information will contribute to the results obtained over all elements.

For the cluster analysis, models without Sr, Pb, Mg, or Mn were significantly concordant (55–85%) with the five-element cluster model, while the model omitting Ba was significantly negatively concordant with the full model (22%, Table 2). Ba, Pb, Mg, and Mn all had higher concentrations in the ‘retained’ cluster relative to the ‘dispersed’ cluster, with variable ranking of the concentrations of the ‘entrained’ cluster (Table 3).

When summing fish classifications across the population, the proportion of fish grouped as R, E, and D were similar for each of the three five-element models (Table 4). The similarity was consistent for groups of fish collected during each sampling month (Table 4). Across months, the proportion of RED fish groupings were variable, with ‘dispersed’ fish overrepresented in August relative to June and July (Table 4). Additionally, the average age at which fish classified as ‘entrained’ from the likelihood model were estimated to have switched among water masses was significantly lower in August relative to June and July (ANOVA, $F_{2,101} = 3.62$, $P = 0.03$, Table 4).

Discussion

For *T. bifasciatum* on the island of St Croix, we analysed trace elemental profiles through time using LA-ICPMS, and

Table 2. Summarised measures of concordance among clustering models

The matrix presents the proportion of fish ($n = 200$) that were identically classified by the two analyses. Three models were tested using all five sampled elements, i.e. maximum likelihood analysis, cluster analysis and Tucker 3-way principal component analysis. For the likelihood and cluster approaches, the analyses were repeated on a subset of four sampled elements, labelled by the one element that was omitted from the analysis

	All elements			Likelihood analyses (four elements)					Cluster analyses (four elements)				
	Likely	Cluster	Tucker	–Sr	–Ba	–Pb	–Mg	–Mn	–Sr	–Ba	–Pb	–Mg	–Mn
All elements													
Likely	–	0.505 ^A	0.520 ^A	0.950 ^A	0.925 ^A	0.965 ^A	0.960 ^A	0.400	0.410	0.275	0.600 ^A	0.345	0.436
Cluster	–	–	0.570 ^A	0.500 ^A	0.475	0.495 ^A	0.480 ^A	0.225 ^B	0.690 ^A	0.220 ^B	0.760 ^A	0.555 ^A	0.845 ^A
Tucker	–	–	–	0.520 ^A	0.505 ^A	0.500 ^A	0.495 ^A	0.290	0.550 ^A	0.365	0.575 ^A	0.475 ^A	0.525 ^A
Likelihood analyses (four elements)													
–Sr	–	–	–	–	0.885 ^A	0.915 ^A	0.930 ^A	0.370	0.435	0.265	0.600 ^A	0.370	0.435
–Ba	–	–	–	–	–	0.950 ^A	0.955 ^A	0.425	0.370	0.240	0.575 ^A	0.325	0.405
–Pb	–	–	–	–	–	–	0.965 ^A	0.415	0.405	0.255	0.595 ^A	0.335	0.425
–Mg	–	–	–	–	–	–	–	0.415	0.385	0.265	0.595 ^A	0.340	0.405
–Mn	–	–	–	–	–	–	–	–	0.240	0.360	0.235 ^B	0.215	0.160 ^B
Cluster analyses													
–Sr	–	–	–	–	–	–	–	–	–	0.365	0.545 ^A	0.725 ^A	0.620 ^A
–Ba	–	–	–	–	–	–	–	–	–	–	0.085 ^B	0.450	0.340
–Pb	–	–	–	–	–	–	–	–	–	–	–	0.435 ^A	0.670 ^A
–Mg	–	–	–	–	–	–	–	–	–	–	–	–	0.460
–Mn	–	–	–	–	–	–	–	–	–	–	–	–	–

^A More identical classifications than expected by chance.

^B Less identical classifications than expected.

Table 3. Mean chemical concentrations estimated by the likelihood and the cluster analyses (standard deviations in parentheses)

Both models fit fish into one of three groups, retained, entrained, and dispersed. Likelihood model fits concentrations from individual pits to either a ‘nearshore’ or an ‘offshore’ water mass, while cluster analysis uses mean chemical concentrations across all pits for an individual fish

		²⁴ Mg : ⁴⁸ Ca (mmol mol ⁻¹)	⁵⁵ Mn : ⁴⁸ Ca (μmol mol ⁻¹)	⁸⁸ Sr : ⁴⁸ Ca (mmol mol ⁻¹)	¹³⁸ Ba : ⁴⁸ Ca (μmol mol ⁻¹)	²⁰⁸ Pb : ⁴⁸ Ca (μmol mol ⁻¹)
Likelihood	‘Nearshore’	0.07 (0.09)	2.00 (1.09)	3.23 (0.47)	0.56 (0.39)	0.27 (0.30)
	‘Offshore’	0.04 (0.07)	0.63 (0.70)	3.53 (0.50)	0.56 (0.39)	0.27 (0.27)
Cluster	‘Retained’	0.05 (0.06)	1.26 (0.59)	3.52 (0.22)	0.84 (0.29)	0.26 (0.57)
	‘Entrained’	0.07 (0.05)	1.54 (0.70)	3.20 (0.19)	0.48 (0.14)	0.27 (0.16)
	‘Dispersed’	0.03 (0.05)	0.98 (0.68)	3.53 (0.18)	0.52 (0.12)	0.16 (0.14)

used a maximum likelihood-based approach to classify fish into three hypothesised dispersal histories: (1) those developing in nearshore waters (i.e. ‘retained’); (2) those developing offshore (i.e. ‘dispersed’); and (3) those showing a mixed profile indicative of a significant switch between two water masses (i.e. ‘entrained’). We compare these results to the RED assignments of other statistical techniques (i.e. cluster analysis, Tucker 3-way PCA). We conclude that the maximum-likelihood approach offers advantages over cluster analysis and Tucker 3-way PCA, despite similarities in the classification of individuals across each technique. Potential information contained in the temporal profile of each element is lost in cluster analysis as it uses only the mean value of

each element across the otolith transect. While Tucker 3-way PCA can use the multi-dimensionality of temporal profiles for multiple elements, it does not explicitly incorporate process uncertainty and machine error in classifying individuals to dispersal histories.

The three statistical techniques compared here present distinct approaches for classifying fish into categories of larval movement. Although the classifications from each model are more similar than expected by chance, 40–50% of fish were classified differently between pairs of models (Table 2). The discrepancy of classifications arises from the distinct assumptions inherent to each model, while the similarities of output may reflect the similarities of the assumptions. A brief

Table 4. Summary of retained/entrained/dispersed classifications under each of three clustering models: the likelihood model from the present paper, three-means cluster analysis of mean chemical concentrations and Tucker 3-way principal component analysis

Retained (R), entrained (E) and dispersed (D) values are the percentage of fish that were classified as retained, entrained, and dispersed across all months and individually for each sampling month. Mean switch is specific to the likelihood model and displays the mean estimated age at which entrained fish switched from the offshore to nearshore water mass. Note that the analyses of fish from August 2001 show a larger representation of dispersed fish for all models and a significantly lower mean switch point of entrained fish in the likelihood model relative to fish in other months. An anomalous current reversal around St Croix also occurred in August

Sampling time	Classification	Likelihood	Cluster	Tucker
Overall (<i>n</i> = 200)	R	21.0	18.0	25.5
	E	52.0	45.0	45.0
	D	27.0	37.0	29.5
	Mean switch	26.0 days		
June 2002 (<i>n</i> = 34)	R	17.6	17.6	32.4
	E	52.9	58.8	52.9
	D	29.4	23.5	14.7
	Mean switch	27.9 days		
July 2002 (<i>n</i> = 96)	R	21.9	19.8	22.9
	E	61.5	53.1	51.0
	D	16.7	27.1	26.0
	Mean switch	26.8 days		
August 2002 (<i>n</i> = 70)	R	21.4	15.7	25.7
	E	38.6	27.1	32.9
	D	40.0	57.1	41.4
	Mean switch	23.1 days		

review of some of these assumptions lends insight into the relative advantages and disadvantages of the different statistical approaches.

Common assumptions of larval movement classification statistics

Two principal assumptions underlie most studies of larval movement using otolith chemistry data: (1) elemental signals in the otolith must reflect fish movement reliably across individuals in the population; and (2) elemental concentrations do not change appreciably with fish age.

Typical approaches using otolith data to determine the movements of reef fish before settlement depend on a critical assumption that fish with similar dispersal histories will yield similar otolith signatures, and that otolith signatures are distinct among fish with different dispersal histories. In the models presented here, we make this assumption explicit by estimating, for each element, a single mean concentration associated with the offshore environment and a single mean associated with the onshore environment; note that for the cluster analysis it is further assumed that the concentrations decrease with distance from land (Bruland 1983), but this is not a requirement of the other models. Consequently,

major changes in water chemistry within either the onshore or offshore environment, whether over space (e.g. as one moves parallel along an island's coastline) or time (e.g. over seasons or years), can invalidate the analysis in the absence of independent, direct measurements of the water chemistry. More generally, if the variability in elemental concentrations between onshore and offshore water masses is small relative to the variability within these two environments, it will not be possible to infer dispersal history. Note that these are not particular shortcomings of the statistical approaches presented here, because no method of analysis based on otolith data alone will be sufficient if one cannot reliably pool information across fish, or if water masses are indistinguishable with respect to the measured elements. In our example, these problems can be partially (but not completely) mitigated by analysing fish collected proximally in time and space, and by measuring otolith concentrations of elements known (or at least strongly suspected) to vary reliably between offshore and nearshore environments of that locale.

Ontogenetic changes of elemental uptake rates are known to exist for many fish (Mugiya and Satoh 1995; Swearer 2000; De Pontual *et al.* 2003). As such, the elemental composition across an otolith transect will be a joint product of changing uptake rates and possible differences in the chemical composition of water masses in which fish are residing. Any study using otoliths as a recorder of the environmental history of a larval fish with a variable larval duration will suffer from the confounding effects of ontogenetic changes. For example, in the data used in the current study an ontogenetic decrease of Sr uptake rate may have obscured an environmental increase in Sr concentrations with proximity to shore.

Specific assumptions of larval movement classification statistics

The three models used in the present paper have distinct assumptions. The cluster analysis assumes that the average chemical composition of the water through which the fish passed is a reasonable proxy for the movement trajectory of the individual. In particular, if the average signature for the elements was high then the fish was classified as retained, if low the fish was classified as dispersed, and if intermediate it was classified as entrained. As such, the cluster analysis should be most effective for systems in which the chemical concentrations decrease close to linearly from shore and in which fish entrainment, if it occurs, happens near the midpoint of the individual's PLD.

The Tucker analysis allows for much more systematic variability of the chemical data. This analysis assumes that each fish has its own mean rate of uptake for all elements and also assumes consistent ontogenetic changes of uptake across all fish. Using these assumptions, the Tucker 3-way PCA detrends much of the data to account for dominant changes of elemental composition. The magnitude of inter-individual rates of elemental uptake in larval fish has yet to be

determined, although this individual-level shifting baseline could cause misclassifications of fish in the RED structure. In particular, it is possible that 'dispersed' and 'retained' fish could be misclassified if a dispersed fish were given an elevated individual-specific uptake rate relative to other fish in the population. Further investigation of the validity of these assumptions is needed.

With our likelihood model, we attempt to make explicit the assumptions driving the statistics. We assume that all elements, if they change with shifting water masses, change at the same time. We assume that all individual fish of a species incorporate elements from the environment at similar rates when exposed to similar conditions. We finally assume that there is no ontogenetic change in elemental uptake rates. These last two assumptions may not be valid, as individuals may differ in their element-specific distribution coefficients and some elements (such as Sr) may regularly change ontogenetically (Mugiya and Satoh 1995; De Pontual *et al.* 2003). To account for this, we have designed this maximum likelihood approach to be flexible by including the capacity to incorporate results from validation studies and thus to increase the accuracy of future model assignments.

The outputs from these models are difficult to test independently, but we can compare the model findings against a priori expectations of likely model outputs. For instance, we are encouraged by the consistent differences in RED classifications across months for the different approaches, which is associated with a seasonal increase in mesoscale eddy activity and corresponding current reversals in the vicinity of St Croix (Harlan *et al.* 2002). We find that the relative frequency of dispersed-classified fish increased in August, while the average time that an entrained-classified fish spent in the offshore water mass decreased (Table 4). During August of 2001, strong current reversals occurred around the entire island immediately preceding an extremely strong pulse of recruitment at Jacks Bay relative to other months (S. L. Hamilton, unpublished data). Swearer (2000) reported that the chemical and physical properties differed between mesoscale eddies and coastal waters of St Croix. In particular, Pb concentrations decreased in the nearshore island wake region at times when current reversals were associated with mesoscale eddy activity. Unfortunately, Swearer (2000) did not measure Mn concentrations from seawater samples in that study, so it is unknown whether nearshore distributions of this element (which drives the maximum likelihood RED classifications) are altered by the propagation of mesoscale eddies near St Croix. Nonetheless, Mn is the only element whose profile differs in shape across the otolith transect among the 3 months of the current study (see Fig. 2b).

The present paper outlines a two-fold strategy to advance the application of otolith chemical analysis to elucidate larval movement patterns. First, we present a novel statistical approach for testing a specific model of larval movement. Our approach specifically treats the variability of LA-ICPMS

chemical data. Second, by designing the model explicitly around system assumptions, we have the capacity to alter the model to account for growing data from validation studies. For instance, the assumptions regarding ontogenetic similarity through time for each element are likely to be false. However, with data showing expected patterns of ontogenetic change through the larval period, the likelihood model could be modified to explicitly account for the direction and magnitude of uptake rates. Through the explicit description of models and their assumptions, statistical tools can be used to advance quickly the results derived from chemical studies. In particular, we highlight how a dialogue between applied studies and validation studies can emerge, with validation studies increasing the accuracy of applied studies, and applied studies highlighting the unresolved assumptions that validation can resolve.

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