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Authors

Thorrold, SR
Jones, GP
Hellberg, ME
et al.

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QUANTIFYING LARVAL RETENTION AND CONNECTIVITY IN MARINE POPULATIONS WITH ARTIFICIAL AND NATURAL MARKERS

Simon R. Thorrold, Geoffrey P. Jones, Michael E. Hellberg,
Ronald S. Burton, Stephen E. Swearer, Joseph E. Neigel,
Steven G. Morgan and Robert R. Warner

ABSTRACT

Quantifying larval retention and connectivity remains a major hurdle in the development of realistic spatially-explicit population models in marine systems. This lack of knowledge is primarily due to the difficulty of conducting mark-recapture studies in species that are characterized by the production of large numbers of small pelagic offspring that suffer high initial mortality rates. Advances in artificial and natural tagging methodologies have, however, significantly increased the ability of marine ecologists to track larvae throughout the pelagic larval phase and subsequent recruitment into benthic populations. Many of these empirical approaches are now possible with the development of DNA sequencing and mass spectrometric instrumentation in the last decade. The presence of artificial tags in recaptured individuals remains the only unequivocal method for marking marine larvae. However, the difficulties of tagging sufficient numbers of larvae, with negligible handling effects, are formidable. Natural tags, including genetic markers and geochemical signatures in calcified tissues, are rarely unique indicators of source location, but have a significant advantage because all larvae released from an area are indelibly tagged. Given the strengths and limitations of the techniques, an approach that combines two or more techniques will likely be necessary to quantify larval retention and connectivity over appropriate spatio-temporal scales. Where possible, such a multi-technique strategy should include both artificial and natural tags.

Many populations of marine organisms are considered to be demographically open whereby recruits to local populations are sourced from a regional pool of reproductive propagules (Roughgarden et al., 1988; Caley et al., 1996). However, there is little direct evidence for high levels of connectivity among marine populations over ecologically-relevant time scales. Exchange of individuals among sub-populations of terrestrial organisms has typically been quantified using mark-recapture approaches (Seber, 1982). Mark-recapture studies are, however, much more daunting in the marine environment. Many marine organisms are highly fecund, often spawning millions of small propagules in a single spawning episode. Tagging these eggs or larvae without introducing significant handling effects is a challenging endeavor. Moreover, extremely high mortality rates of early life history stages mean that the probability of recovering a tagged individual is extremely small. These impediments are directly responsible for the lack of empirical data on larval retention and connectivity in marine populations.

Despite the inherent difficulty of mark-recapture studies in marine populations, considerable efforts have recently been devoted to the development of new tagging strategies for use in aquatic systems (Levin et al., 1993; Jones et al., 1999; Swearer et al., 1999). These efforts are necessary if spatially-explicit models are to provide useful facsimiles of the population dynamics of marine organisms. Here, we critique the empirical approaches available for quantifying connectivity in marine populations. The concepts behind many

of the approaches are not particularly novel, but the information available from these methods has rapidly increased with the development and subsequent refinement of molecular genetic techniques and mass spectrometric instrumentation in the last decade. We conclude by highlighting the significant advantages gained by the use of multiple tagging approaches in marine environments.

GENERAL PROPERTIES OF POPULATION MARKERS

The design of an effective study for quantifying connectivity in marine populations is obviously contingent upon the availability of appropriate marking technology. To be successful in the marine environment, a tagging method must have several characteristics; some of these are common to all mark-recapture studies, while others are particularly pertinent in marine systems.

1. Marked individuals must be able to be unambiguously identified some time after application of the tag. This assumption requires tag retention for an appropriate length of time, and that all marked individuals are recognized and recorded.
2. Mortality rates and behavior of tagged organisms must be indistinguishable from those of the larger untagged population. Tags must therefore be invisible to predators, non-toxic and have no other potential effects on subsequent growth and survival of marked individuals.
3. Tagging approaches must be able to mark large numbers of individuals, preferably in a cost-effective manner. Extremely high mortality rates during pelagic larval life means that few tagged larvae will eventually survive to recruit back into adult populations.
4. Finally, because tagged individuals will generally make up a very small proportion of the total number of individuals collected, the mark must be relatively quick and inexpensive to detect.

These requirements have led to the development of several different approaches to mark-recapture studies in marine systems that are appropriate for quantifying connectivity. The simplest and least ambiguous method relies upon artificial tagging of organisms by the researcher. Genetic markers take advantage of natural variation in gene frequencies among source populations, or may be based on genetic tags generated by artificial breeding. Finally, environmental tags rely upon natural variation in the physico-chemical conditions experienced by individuals from different locations to leave an indelible mark of water mass residency. We outline each of the approaches below, with examples drawn from the literature where available.

ARTIFICIAL TAGS

Direct visual observation has been used successfully to track dispersal of a few invertebrates with extremely large larvae (e.g., Olson and McPherson, 1987), but has limited applicability to most marine species. Miniaturized physical tags can be applied to post-settlement juveniles in many marine taxa (e.g., Buckley et al., 1994). However, these tags are not small enough to be applied to early larval stages that typically hatch at sizes less than 5 mm. Two categories of artificial marks can potentially meet the criteria for routine use in larval mark-recapture studies in marine systems. The first involves the immersion of embryos or larvae in marker chemicals that are incorporated into body tissues. Chemi-

cal marks used for this purpose include fluorescent compounds, elemental tags, and radioactive isotopes. The second approach is based on physical marks in structures that are formed by environmental perturbations, such as thermal signals.

FLUORESCENT COMPOUNDS

Fluorescent compounds are readily incorporated into calcified tissues of larvae such as bone or shell. They have been successfully used to mark larvae of a number of fish species, particularly anadromous species or those confined to enclosed estuaries (Table 1). Fluorescent labels are easy to apply to large numbers of larvae by immersion and have been successfully employed to mass mark batches of fish larvae in the field (Jones et al., 1999). They have also been successfully applied to invertebrate taxa with calcified structures, including articulate brachiopods, molluscs, echinoderms, bryozoans, polycheates and crustaceans (Loosanoff and Davis, 1947; Rowley and Mackinnon, 1995; Moran, 2000). The most widely tested compounds include alizarin complexone, alizarin red S, tetracycline and its derivatives, and calcein. Calcein appears to be particularly suitable for invertebrates (Rowley and Mackinnon, 1995; Moran, 2000). Each compound produces fluorescence of a different color under light of a specific wavelength (Fig. 1). Combinations may be achieved by multiple marking with several compounds (Tsukamoto, 1988), or with the same compound when repeat marking is spaced by several days (Dabrowski and Tsukamoto, 1986).

Tagging using fluorescent compounds offers a number of advantages over other methods. Larvae can be marked at the egg or embryo stage, prior to any skeletal development (e.g., Jones et al., 1999). The technique is robust, with a number of studies achieving almost 100% marking efficiency over a wide range of chemical concentrations, immersion times and development stages (Table 1). Studies have shown that marks last well beyond metamorphosis (often 100s of days), making them potentially useful for tracking the larvae of marine organisms with long pelagic larval durations (Table 1). Importantly, the growth and mortality of marked larvae appear to be similar to unmarked larvae (Secor et al., 1995; Jones et al., 1999; Moran, 2000; but see Brooks et al., 1994; Mohler, 1997). The main disadvantage of fluorescence marking, at least in fish, is that the tag cannot be detected externally. Large numbers of juveniles must be sacrificed and bony structures removed and examined in order to detect marks. Detection of fluorescent labels in larval shells of gastropods is, however, possible under a dissecting microscope without any sample preparation (Moran, 2000).

ELEMENTAL TAGS

Recent efforts have focussed on elemental tags for larval invertebrates and fishes. The rationale is that such elements occur at low levels in natural environments, so any larvae with these signatures will be of known origin. As with fluorescent chemicals, elemental tags are easily applied to large batches of larvae. Marked juveniles can be readily detected using standard spectrometric instruments that target the core of the calcified structure where marks are located. However, the costs associated with preparation and analysis are often high relative to fluorescent marking techniques.

Strontium has been the most widely used element for marking larval and juvenile fishes. Immersion in strontium chloride is known to produce detectable and long-lasting signatures in scales, otoliths, vertebrae, opercula and dorsal spines in juveniles of a variety of fishes (Table 1). The main problem with strontium is that it is found naturally in fishes,

Table 1. Marking of teleost fishes by fluorescent, elemental, and radionucleotide methods. Fluorescent chemicals are oxytetracycline (OTC), alizarin complexone (AZC), and alizarin red (AZR). Optimal immersion times, chemical concentrations, mark longevity, developmental stage (embryos [E], larvae [L], or juveniles [JJ], and methods of detection (ultraviolet [UV] microscopy, high performance liquid chromatography [HPLC], wavelength dispersive electron probe microanalysis [EPMA], and inductively coupled plasma mass spectrometry [ICP-MS]) are provided.

Chemical	Immersion time	Concentration	Mark longevity	Stage	Method of detection	Reference
FLUORESCENT						
OTC	1–2 hr	250–500 mg L^{-1}		L	UV microscopy	Hettler (1984)
OTC	24–48 hr	200–300 mg L^{-1}	105 d	E	UV microscopy	Tsukamoto (1985)
	3–24 hr	200–300 mg L^{-1}		L		
OTC	12 hr	600 mg L^{-1}	30–90 d	E	UV microscopy	Dabrowski and Tsukamoto (1986)
	3–6 hr	600 mg L^{-1}		L		
	35 hr	300 mg L^{-1}		J		
Calcein	2 hr	125 mg L^{-1}	1 yr	L and J	UV microscopy	Wilson et al. (1987)
AZC	24 hr	50–200 mg L^{-1}		E and J	UV microscopy	Tsukamoto (1988)
OTC		200–300 mg L^{-1}				
Calcein		500 mg L^{-1}				
OTC	2–3 hr	0.25–0.35 mg L^{-1}	180 d	L	Epifluorescence and UV microscopy	Secor et al. (1991)
OTC	6 hr	500 mg L^{-1}	1.25 yr	L	UV microscopy	Brooks et al. (1994)
Calcein				J		
Calcein blue						
OTC	3.5–13 min	1%	1 yr	E	Fluorescence Microscopy	Beltran et al. (1995)
	1.5 min	1%		L		
	<1 min	1%		J		
AZR	12–24 hr	200–300 mg L^{-1}	180 d	L and J	Bright-field Microscopy	Beckman and Schulz (1996)
AZC						
OTC	6 hr	447 mg L^{-1}	240 d	J	UV microscopy	Bumguardner and King (1996)
Calcein		125 mg L^{-1}				
OTC	48 hr	250 mg L^{-1}	240 d	L	UV microscopy	Mohler (1997)
Calcein	48 hr	125 mg L^{-1}		L		
OTC	12 hr	300–750 mg L^{-1}	105 d	J	HPLC, fluorescence Microscopy	Unkenholz et al. (1997)
OTC	6–8 hr	350–400 mg L^{-1}	3 yr	L	Epifluorescence Microscopy	Reinert et al. (1998)
OTC		400 mg L^{-1}	120–180 d	L and J	UV microscopy	Nagiec et al. (1988)

Table 1. Continued.

Chemical	Immersion time	Concentration	Mark longevity	Stage	Method of detection	Reference
ELEMENTS						
$SrCl_2$	60 d	75–250 mg L^{-1}	240 d	J	X-ray fluorescence Spectrometry	Behrens Yamada et al. (1979)
$SrCl_2$	60 d	75–250 mg L^{-1}	1.5 yr	J	Leaching Technique	Behrens Yamada and Mulligan (1982)
$SrCl_2$	50	1 g L^{-1}	180 d	L	Leaching Technique	Behrens Yamada and Mulligan (1987)
$SrCl_2$	30–60 d	1.8–5.4 mg L^{-1}		J	ICP-MS	Synder et al. (1992)
$La(C_2H_3O_2)_3$ $Ce(C_2H_3O_2)_3$ $LaCl_3$ and $CeCl_3$	24 d	50 mg L^{-1}		J	ICP-MS	Ennevor (1993)
$La(C_2H_3O_2)_3$ $Sm(C_2H_3O_2)_3$	28 d	0.2 mg L^{-1}		J	ICP-MS	Ennevor and Beames (1993)
Dy and Eu Sm	Injection	12– 254 g L^{-1}	1.5 yr 2 yr	J	Neutron activation Analysis	Giles and Attas (1993)
$SrCl_2$	24 hr	0.1– 9 mg g^{-1}	1.7 yr	J	EPMA ICP-MS	Schroder et al. (1995)
$SrCl_2$	5 d	250 mg L^{-1}	36 d	J	ICP-MS	Pollard et al. (1999)
RADIOACTIVE ISOTOPES						
^{85}Sr	7 d	6.4– 16.8 µCi		L	Ge-Li gamma-ray detector	Carlson and Shealy (1972)
Eu	3 hr	37 m Bq	3.5 yr	J		Hansen and Fattah (1986)
^{85}Sr	2 d	1400 kBq L^{-1}	60 d	L	Radioactivity	Lehtonen et al. (1992)
^{85}Sr		1.85 mBq L^{-1}	28–42 d	L	Radioactivity	Vuorinen et al. (1998)

with tissue concentrations varying in different environments as a function of water chemistry, temperature, and diet (reviewed by Campana, 1999). Hence, to validate the use of strontium as a tag, it is necessary to show that all marked larvae retain a significantly higher proportion of the element than unmarked fish (Pollard et al., 1999).

Elemental tagging using rare earth elements (REEs) has also been tested on juvenile salmonids. REEs occur naturally in the bones of many fishes at extremely low concentrations (low ng g^{-1}, S. R. Thorrold, pers. observ.), which makes them less ambiguous markers than strontium. Most are non-radioactive, easy to handle and have long residence times in bony tissues. For example, Ennevor and Beames (1993) found that lanthanum and cerium tags in juvenile salmon last at least 10 mo. Similarly, detectable levels of dysprosium, europium and samarium lasted at least 2 yrs in rainbow trout, with levels of samarium remaining unchanged over this period (Giles and Attas, 1993). Comparisons of treated and untreated juveniles have not detected any deleterious effects on growth, survival or reproductive development (Giles and Attas, 1993; Ennevor, 1993).

Several studies have attempted to mark invertebrate larvae using trace elements and REEs (Levin, 1990). Most of the elements tested in laboratory studies were either not absorbed, or were not retained for a sufficient length of time to serve as a tag, even in larvae that deposited calcified shell material (Levin et al., 1993). A recent laboratory study found selenium to be a suitable marker for larval invertebrates (Anastasia et al., 1998). Mass-marking in the field may be possible if elements are passed from reproductive females to larvae. Millions of offspring could then be marked by dusting a prescribed area with the element, as has been achieved with insects (Hayes, 1991).

RADIOACTIVE ISOTOPES

Radioactive isotopes offer another promising method of marking the larval stages of marine organisms. However, due to significant safety issues, only a few radionuclides are potentially useful for marking embryos or yolk-sac larvae. Beta-emitters (e.g., ^{45}Ca, ^{89}Sr) are not feasible, as detection requires extensive laboratory preparation (Lehtonen et al., 1992). It is environmentally unacceptable to use radionuclides with long half-lives (e.g., ^{90}Sr), even though they would be excellent markers. Most fish studies have used ^{85}Sr, a gamma-emitter which can easily be detected with minimal sample preparation (Table 1). The half life of ^{85}Sr (65 d) is appropriate for examining larval dispersal in most marine species, without generating any long-term environmental problems. Studies on pike (*Esox lucius*) indicate that ^{85}Sr marked larvae can be distinguished from unmarked juveniles after 4–6 wks, without any deleterious affects on growth or survival (Vuorinen et al., 1998). Another gamma-emitting radioisotope (^{65}Zn) was recently used to confirm homing of adult mysids (*Mysidium gracile*) to swarm sites after nocturnal dispersal into the water column in Discovery Bay, Jamaica (Twining et al., 2000). Although the half life of ^{65}Zn is also appropriate for examining larval dispersal (244 d), it is not known if Zn turnover rates are slow enough to allow for recovery of the radioisotope over a period of weeks to months.

THERMAL MARKS

Thermal marking of otoliths is commonly used for examining the fate of hatchery-produced salmonids (Fig. 1), and may have wider applications to marine organisms. The procedure involves exposing incubating larvae to short-term temperature fluctuations that result in distinctive structural banding on the otoliths (Volk et al., 1999). Periodic
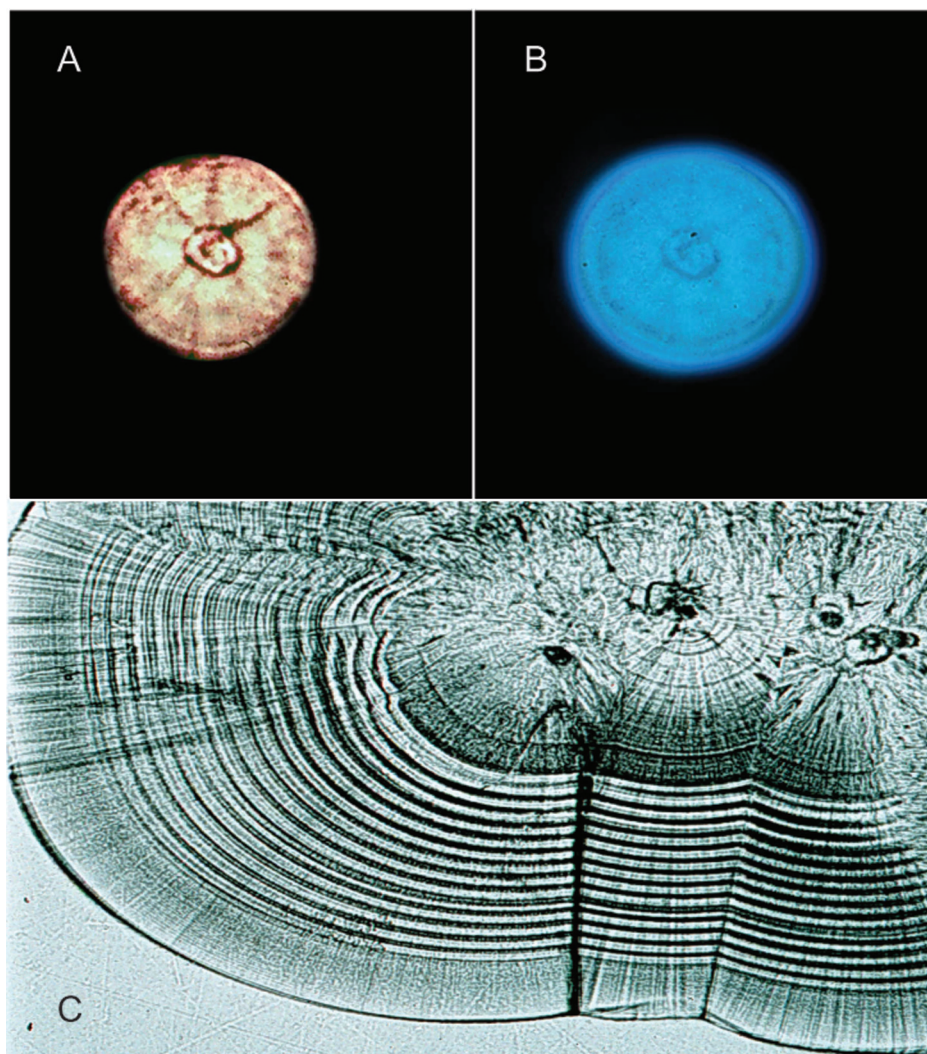
Figure 1. Upper panels: Photomicrographs of an otolith from the embryo of a coral reef fish, *Pomacentrus amboinensis*, marked with oxytetracycline viewed under transmitted standard light (A) and UV light (B). Lower panel (C): Photomicrograph of thermally-induced marks in the sagittal otolith of a juvenile coho salmon (*Oncorhynchus kitusch*). A total of 16 exposures were applied to the juvenile coho (yellow arrows mark first and last exposure), each of two days at ambient temperatures (dark zones in otoliths) or at temperatures elevated 2.5°C above ambient (light zones in otolith). Photograph courtesy of Dr. E. Volk.

exposure of embryos to chilled water (4ᵒC) produces a much greater contrast between light and dark zones in daily otolith increments (Volk et al., 1990). Changes in the periods of exposure to different water temperatures alter the widths of increments. Therefore, unique codes can be established by manipulating the frequency and duration of periods of immersion in chilled water. This gives thermal marking considerable flexibility in producing different batch marks. Thermal mark recovery is achieved by visual examination of otoliths, which is costly in terms of labor.

In summary, chemical and thermal marking provide several possible options for marking the embryos of marine organisms to examine larval dispersal. All are promising, but require further development and testing on fully marine species, and in physically energetic environments. Each of the techniques is relatively easy to apply and has the potential to be applied en masse, but field trials are likely to be time-consuming and expensive. As with any mark-recapture approach, each of the marking procedures requires the recapture and detection of a sufficient number of tagged individuals from which to draw valid conclusions (Seber, 1982). In the one successful field trial to date, approximately 1% of the population was marked and only 15 marked individuals were retrieved (Jones et al., 1999). Clearly, to get reliable estimates of self-recruitment, a significant proportion of the larval production must be tagged and a large proportion of subsequent recruitment to a population must be collected. The prospects of quantifying connectivity in marine environments using these methods seem rather remote until methods are developed to target marked juveniles specifically.

NATURAL TAGS

The difficulties associated with traditional mark-recapture studies have led to considerable interest in the application of natural tags in marine systems. Naturally-occurring markers are of particular interest to marine ecologists because they eliminate the need for the researcher to tag an individual physically. Rather, they rely upon genetic heterogeneity or differences in environmental conditions to generate population-specific markers. The interpretation of data from natural tags is almost invariably more equivocal than that of mark-recapture studies using the physical tags described in the preceding section. However, since every individual from a population is marked, natural tags are particularly useful when significant larval dispersal and high mortality reduce the likelihood of recovering physical tags.

GENETIC MARKERS

The extent of genetic differentiation among marine populations is often extremely small (Hellberg et al., this issue). Several tagging approaches based on genetic markers are available that do not require significant differentiation at the population level, but rather rely upon natural variation in spawning success, or laboratory propagation of rare natural or transgenic markers.

Natural Cohorts.—Genetic insights into present-day population connectivity are most readily accomplished with sampling programs that include new recruits. A number of studies have demonstrated that natural cohorts of marine organisms are sometimes genetically distinct form either subsequent cohorts, or from the adult population into which they have recruited (e.g., Koehn et al., 1980; Kordos and Burton, 1993; Edmands et al., 1996; Moberg and Burton, 2000). Several processes might contribute to such recruit diversity: (1) recruits may be derived from different source populations, (2) natural selection occurring before the cohort is sampled may favor different genotypes in space and time, and (3) recruits may be derived from limited subsets of parents.

Regardless of how cohorts of recruits become genetically distinct, can we take advantage of such natural genetic variation to make inferences about patterns of larval retention and population connectivity? The answer is a qualified 'yes'. If the observed genetic variation is generated by pre-settlement natural selection, then the result is a function of

differential mortality rather than reproductive isolation. Although it is difficult to distinguish between natural selection and reproductive isolation scenarios, analysis of multiple genetic markers will often permit strong inference regarding the likelihood of the selection scenario. For example, Kordos and Burton (1993) found that three markers showed similar patterns of temporal change; it is unlikely that selection would act similarly on three unlinked genetic loci.

Given the different mechanisms by which genetic variation in recruits can be generated, what are the prospects for research programs employing such an unpredictable tool? Certainly if the sampled cohorts are genetically homogeneous in space and time (like adults), little information is gained from recruit analysis. However, genetic sampling of recruits from several highly dispersive invertebrate systems has provided more insight into population connectivity than could be gleaned from studies of adults alone (Koehn et al., 1980; Kordos and Burton, 1993; Edmands et al., 1996; Moberg and Burton, 2000). Even though such studies will rarely be able to determine larval sources, they may provide useful information linking spatio-temporal patterns of recruitment and population connectivity.

Artificial Breeding.—Artificial breeding can be used to increase the frequency or homozygosity of genetic variants to levels sufficiently different from natural frequencies for individuals receiving these genetic tags to be recognized upon recapture. This can be done either by creating individuals that are homozygous for rare alleles, or by crossing individuals that carry multiple rare alleles at hypervariable loci to create discernable multi-locus genotypes. Artificial genetic markers are assumed to be selectively neutral, as with any tag. However, close inbreeding may be required to create homozygous nuclear markers. Inbreeding generally has strong, negative fitness consequences in normally outbreeding organisms, including marine species with pelagic larvae (C. Langdon and D. Hedgecock, pers. observ.). The interpretation of recovered individuals with genotypes identical to the artificial genetic tag during the recapture process also requires caution. A false positive recovery may be obtained from a pseudotagged individual created by natural interbreeding between wild parents that share the rare marker allele or alleles. Predicting the frequency at which such pseudotagged individuals will occur requires assumptions about mate choice, effective population size, and the degree of population connection, which are exactly the parameters that one might hope to estimate from a tagging study. Moreover, while multiple hypervariable loci can assign individuals to particular populations of origin with quite high (90–95%) probability (Bernatchez and Duchesne, 2000), this level of accuracy may still be insufficient to resolve questions of connectivity where only low to modest levels of migrations are expected.

The overall effort required to tag individuals by artificial breeding is considerable. Maternally inherited mtDNA offers a large practical advantage in this regard because all offspring of a mother with a rare mitochondrial haplotype will share that marker without being subjected to reduced heterozygosity at nuclear loci (Wilson et al., 1997). Many marine species are not amenable to artificial breeding, and rearing is often hardest for those species with extended pelagic development stages where dispersal may be particularly important. On the other hand, captive populations that have been artificially bred for aquaculture may become incidentally tagged in the process (e.g., Perez-Enrique and Tanigushi, 1999). Ascertaining the genotype of recaptured individuals can also be both expensive and time consuming. Such sampling is generally destructive for most small species. Pilot studies have, however, demonstrated that minimally invasive techniques

can be used to survey hundreds of artificially tagged individuals (Wilson and Donaldson, 1998).

To date, artificial breeding for genetic tagging has been assessed primarily as a means of evaluating stock enhancement programs (Kristiansen et al., 1997; Wilson and Donaldson, 1998; Perez-Enrique and Tanigushi, 1999). An inbred genetic tag has, however, been used to trace dispersal of larvae and sperm in a colonial tunicate (Grosberg, 1991), although this proved fruitful only over small spatial scales (<1 m).

Transgenic Individuals.—In theory, novel DNA sequences can be inserted into animals using transgenic technology, thereby producing a genetic marker that will not only last for the lifetime of the genetically modified organism, but should be passed to future generations as well. Transgenic markers can be completely unlike anything found in nature, thereby eliminating the possibility of recovering pseudotagged individuals. Furthermore, transgenic tagging holds the possibility of virtually eliminating timely and costly genetic analysis during the recapture phase of study by introducing reporter genes that produce clear visual tags such as green fluorescent protein (Gibbs and Schmale, 2000). The development of transgenic animals remains, however, both technically challenging and expensive (Levy et al., 2000).

The biggest assumption when using transgenic individuals for quantifying connectivity is that the mortality and dispersal capabilities of marked larvae are similar to those of unmodified individuals. In laboratory experiments, Abrahams and Sutterlin (1999) found that transgenic Atlantic salmon (*Salmo salar*) that had received a growth hormone were characterized by far higher growth rates and average swimming speeds than control groups. Even a transgene whose product is selectively neutral may alter survival of individuals by disrupting the genome where it is inserted.

Given the total research effort that has been invested, the introduction of transgenic material into genomes of aquatic organisms has been reasonably successful. For example, a transgenic goldfish was among the first successful gene transfers in animals (Zhu et al., 1985). The external fertilization and high fecundity shared by many aquatic animals may facilitate gene-introduction approaches unavailable in internally fertilized animals. Retroviral vectors with broad host ranges offer the promise of gene insertion in a wide variety of marine organisms, even those with limited research histories (Lu et al., 1996). Thus, the prospect of practical tagging with transgenes remains real, but the environmental consequences of such an approach warrant close attention (Zilinskas and Balint, 1998; Muir and Howard, 1999; Hedrick, 2001).

ENVIRONMENTAL MARKERS

Another natural tag relies upon larvae experiencing variations in the physical and chemical environments that generate phenotypic variations in larval form or growth rate. Alternatively, variability in environmental exposure may be recorded in the trace element or isotope chemistry of skeletal or other hard material. Trace elements in coastal waters vary due to natural and anthropogenic causes, and this variation may in turn impart distinct elemental signatures to larvae that develop within different locations. Environmental markers are generally much less sensitive to migration than standard population genetic approaches, as environmental conditions experienced by larvae are not generally passed on to subsequent generations. However, this lack of temporal stability implies that ground-truthing of environmental tags will generally need to be conducted on every cohort of interest.

Developmental Markers.—Larvae recruiting to local marine populations can often exhibit strong intraspecific variation in size and condition. Such variation can result from differences in maternal provisioning, larval food availability or water temperature. In some circumstances, these effects on larval size and condition may vary predictably among local populations, providing a developmental tag of larval origin. For instance, Gaines and Bertness (1992) found large differences in size of competent larvae of the barnacle *Semibalanus balanoides* between bay and open ocean populations. Berghahn and Karakiri (1990) were able to induce marks in otoliths of plaice (*Pleuronectes platessa*) by altering water temperature, levels of UV-B radiation, and feeding regimes. Similar marks were found in otoliths of plaice and flounder from natural populations in the North Sea (Berghahn, 2000), suggesting that geographic variability in exposure to environmental stresses may generate location-specific natural tags in otoliths of some North Sea flatfish. Swearer et al. (1999) used a combination of larval growth rates and trace element signatures in otoliths to quantify local retention of larvae of the bluehead wrasse (*Thalassoma bifasciatum*) in the vicinity of St. Croix, US Virgin Islands.

In addition to variation in larval size, differences in larval growth history can also result in either meristic or morphological variation. For example, fish larvae often show temperature-dependent effects on the development of meristic characters such as the number of vertebrae and fin rays and spines (Fowler, 1970; Murray and Beacham, 1988). Even differences in temperature history between spawning females can cause meristic differences in their progeny (e.g., Swain and Lindsey, 1985). Such effects at the local population scale could therefore provide an environmental tag associated with temperature differences or habitat associations among source populations (Swain and Frank, 2000).

Developmental tags may have limited application because they rely upon a priori knowledge of spatial variation in the biological (e.g., food resources) and physical (e.g., temperature) factors that result in differences in larval morphology among source populations. In addition, these factors often exhibit great spatial and temporal variability (Denman and Powell, 1984) and therefore may not result in consistent 'marking' of larvae spawned from the same population. Finally, these types of markers are likely to be relatively short-lived as variation in developmental traits can disappear rapidly after settlement (e.g., loss of larval morphologies, rapid and highly variable post-settlement growth).

GEOCHEMICAL SIGNATURES IN CALCIFIED STRUCTURES.—Natural tags of population origin may reside in the trace element and isotope chemistry of calcified structures in both invertebrate and fish larvae. Much of the initial work in the field has been conducted on fish otoliths, which are in many ways particularly well suited for this application. The use of geochemical signatures in otoliths as a natural tag requires that there be sufficient spatial differences in the water chemistry to generate unambiguous signatures of water mass residency. It is perhaps not surprising, therefore, that initial work has focused on estuarine and coastal systems, which are characterized by more spatial variation in water chemistry than typically found in open ocean environments (Gillanders and Kingsford, 1996; Thorrold et al., 1998).

In more ambitious applications, two recent studies have examined the feasibility of using otolith geochemical signatures as natural tags in coral reef fishes. Dufour et al. (1998) found that stable carbon and oxygen isotopes in the otoliths of *Chaetodon ulientensis* and *Acanthurus triostegus* collected in the enclosed lagoon of Taiaro Atoll, French Polynesia, were significantly different from individuals collected on the outer reef slope. It would, therefore, be relatively easy to determine if recently-settled juveniles spent their

entire life within the lagoon based on these isotopic signatures. Swearer et al. (1999) found that near-coastal waters in the vicinity of the island of St. Croix were characterized by higher dissolved concentrations of Mn, Ba and Pb than oceanic waters in the same region, and suggested that these differences were accurately recorded in the otolith chemistry of newly recruited *Thalassoma bifasciatum*. These data indicated that trace element signatures in otoliths were sensitive to relatively subtle differences in water chemistry, suggesting that the technique may be widely applicable in marine systems.

Natural tags based on geochemical signatures in calcified structures also show considerable promise for tracking invertebrate larvae. In the first application of its kind, DiBacco and co-workers (DiBacco and Levin, 2000; DiBacco and Chadwick, 2001) traced the origins of stage I larvae of the striped shore crab, *Pachygrapsus crassipes*, over a single spring tidal cycle in San Diego Bay, California. Larvae spawned inside the bay were distinguished from those spawned on the open coast by determining trace element signatures (Ca, Mn, Zn, Cu and Sr) in the zoeal skeletons. This technique also holds considerable promise for gastropod and bivalve larvae that maintain calcified structures throughout the planktonic period.

Geochemical signatures have several disadvantages compared to conventional tagging studies. Natural tags are rarely unequivocal, and often require sophisticated multivariate algorithms for source assignments (e.g., Thorrold et al., 1998). Some of this ambiguity may be avoided in the future with the development of more robust signatures. For instance, stable isotopes of several elements (e.g., Sr) in otoliths show considerably lower levels of among-fish variation, and higher correlation with values in the ambient environment, than corresponding element/Ca ratios (Kennedy et al., 1997; Kennedy et al., 2000). Another limitation of the technique is that ground-truthed signatures must be available from all potential, or at least likely, source locations. Finally, instrumentation for quantifying geochemical signatures, such as ICP-MS, is expensive and requires considerable analytical expertise to produce accurate data.

Synthesis

Estimating larval retention and connectivity in marine systems is undoubtedly a difficult proposition. Probabilistic modeling approaches based on physical oceanographic observations have yet to provide satisfying answers. We know enough about larval behavior to reject assumptions of larvae as passive particles, but have insufficient information to characterize either larval behavior or fine-scale hydrographic phenomena with the precision necessary to parameterize such models. Methods that take advantage of inherent genetic variation at the population level can make a powerful case for a high degree of isolation (Hellberg et al., this issue). Put another way, these techniques determine the spatial scale at which populations are closed over evolutionary time scales. However, they are less useful in determining connectivity among populations when there is some level of exchange over ecological time. Accurate and robust estimates of larval retention and connectivity will require a direct approach based on mark-recapture techniques. What, then, might be the best protocol to follow? Certainly the optimum approach will necessarily depend on the species of interest and the environment in which the organism lives. Nonetheless, some useful generalizations are possible.

We believe that too little attention has been paid to the potential benefits of multi-technique approaches to the problem of estimating larval retention and population con-

nectivity. Perhaps the easiest multi-technique approach to implement would include two natural markers. For instance, it may be possible to quantify location-specific, but ephemeral, genetic differences among eggs or early-stage larvae produced by sweepstake effects during spawning events (e.g., Li and Hedgecock, 1998). Ground-truthed geochemical signatures in calcified structures could presumably be obtained from the same biological samples. Providing significant differences among locations where present in the both ground-truthed data sets, genetic and geochemical analyses of recent recruits from the same locations may allow independent estimates of connectivity.

There is, of course, no guarantee that different tagging approaches will generate concordant results. Thorrold et al. (2001) used geochemical signatures in otoliths to examine population connectivity through homing to natal estuaries of weakfish (*Cynoscion regalis*). They found natal homing rates as high as 80%, even though population genetic analyses of the same fish using microsatellite and intron loci detected no significant geographic structure (Cordes, 2000). These results are not in conflict, as there was sufficient straying of spawning weakfish to prevent genetic differentiation among estuaries. The study did, however, serve to highlight the power of a natural geochemical tag to provide estimates of connectivity in the absence of detectable genetic heterogeneity.

A more useful multi-technique study might combine both artificial and natural tags. Data from the artificial tags can then be used to validate estimates from the natural tags. In this way a subset of individuals from a single source location, tagged with a fluorescent or elemental marker, could be used to determine the accuracy of a natural marker applied over larger spatial or longer temporal scales. For instance, stock associations obtained independently from coded wire tags have been used to determine the accuracy of mixed stock analysis (MSA) using allozymes in chinook salmon (*Onchorynchus tshawytscha*). Stock proportions based on the genetics data had absolute errors of less than 3% (Brodziak et al., 1992), suggesting that the MSA approach was sufficiently accurate for routine determinations of stock associations in the commercial fishery. More recently, Kennedy et al. (2000) released genetically-marked Atlantic salmon (*S. salar*) fry in a small stream to determine intra-population variability of Sr isotope signatures in vertebrae. Although neither study specifically addresses questions of population connectivity, researchers using natural tags to address these issues would clearly benefit from similar validation studies.

In conclusion, mark-recapture studies of early-stage marine fish and invertebrates present formidable challenges. The difficulties of marking sufficient numbers of larvae may be alleviated by the development of a tag that could be transferred from the female to developing eggs or embryos. Alternatively, new genetic tagging approaches may be needed that retain the advantages of transgenic markers without the environmental risks. In either case, low recovery rates will likely mean that the approach will not work if dispersal distances are large or connectivity among numerous source locations is high. Tags such as genetic analyses of natural cohorts or geochemical signatures in calcified structures appear to be the only realistic alternatives. Ultimately, rates of larval retention and connectivity in marine populations will determine patterns of colonization, the resiliency of species to human exploitation, and the design of marine protected areas. Given this importance, we urge that every effort be taken to obtain independent corroboration of connectivity estimates from such studies.

LITERATURE CITED

Abrahams, M. V. and A. Sutterlin. 1999. The foraging and antipredator behaviour of growth-enhanced transgenic Atlantic salmon. Anim. Behav. 58: 933–942.

Anastasia, J. C., S. G. Morgan and N. S. Fisher. 1998. Tagging crustacean larvae: assimilation and retention of trace elements. Limnol. Oceanogr. 43: 362–368.

Beckman, D. W. and R. G. Schulz. 1996. A simple method for marking fish otoliths with alizarin compounds. Trans. Amer. Fish. Soc. 125: 146–149.

Behrens Yamada, S. and T. J. Mulligan. 1982. Strontium marking of hatchery reared coho salmon, *Oncorhynchus kisutch* Walbaum, identification of adults. J. Fish Biol. 20: 5–9.

_____ and T. J. Mulligan. 1987. Marking nonfeeding salmonid fry with dissolved strontium. Can. J. Fish. Aquat. Sci. 44: 1502–1506.

_____, _____ and S. J. Fairchild. 1979. Strontium marking of hatchery-reared coho salmon (*Oncorhynchus kisutch*, Walbaum). J. Fish Biol. 14: 267–275.

Beltran, R. R., A. Champigneulle and G. Vincent. 1995. Mass-marking of bone tissue of *Coregonus lavaretus* L. and its potential application to monitoring the spatio-temporal distribution of larvae, fry and juveniles of lacustrine fishes. Hydrobiologia 300: 399–407.

Berghahn, R. 2000. Response to extreme conditions in coastal areas: biological tags in flatfish otoliths. Mar. Ecol. Prog. Ser. 192: 277–285.

_____ and M. Karakiri. 1990. Experimental induction of biological tags in otoliths of 0-group plaice *Pleuronectes platessa* by starvation, temperature and UV-B radiation. Mar. Ecol. Prog. Ser. 67: 227–233.

Bernatchez, L. and P. Duchesne. 2000. Individual-based genotype analysis in studies of parentage and population assignment: how many loci, how many alleles? Can. J. Fish. Aquat. Sci. 57: 1–12.

Brodziak, J., B. Bentley and D. Bartley. 1992. Tests of genetic stock identification using coded wire tagged fish. Can. J. Fish. Aquat. Sci. 49: 1507–1517.

Brooks, R. C., R. C. Heidinger and C. C. Kohler. 1994. Mass-marking otoliths of larval and juvenile walleyes by immersion in oxytetracycline, calcein, or calcein blue. North Amer. J. Fish. Manag. 14: 143–150.

Buckley, R. M., J.E. West and D.C. Doty. 1994. Internal micro-tag systems for marking juvenile reef fishes. Bull. Mar. Sci. 55: 848–857.

Bumguardner, B. W. and T.L. King. 1996. Toxicity of oxytetracycline and calcein to juvenile striped bass. Trans. Amer. Fish. Soc. 125: 143–145.

Caley, M. J., M. H. Carr, M. A. Hixon, T. P. Hughes, G. P. Jones and B. A. Menge. 1996. Recruitment and the local dynamics of open marine populations. Annu. Rev. Ecol. Syst. 27: 477–500.

Campana, S. E. 1999. Chemistry and composition of fish otoliths: pathways, mechanisms and applications. Mar. Ecol. Prog. Ser. 188: 263–297.

Carlson, C. A. and M. H. Shealy, Jr. 1972. Marking larval largemouth bass with radiostrontium. J. Fish. Res. Bd. Can. 29: 455–458.

Cordes, J. F. 2000. Application of genetic markers to provide species identification and define stock structure: analyses of selected marine species of the mid-Atlantic Bight. Dissertation, College of William and Mary, Williamsburg, Virginia.

Dabrowski, K. and K. Tsukamoto. 1986. Tetracycline tagging in coregonid embryos and larvae. J. Fish Biol. 29: 691–698.

Denman, K. L. and T. M. Powell. 1984. Effects of physical processes on planktonic ecosystems in the coastal ocean. Oceanogr. Mar. Biol. Ann. Rev. 22: 125–168.

DiBacco, C. and L. A. Levin. 2000. Development and application of elemental fingerprinting to track the dispersal of marine invertebrate larvae. Limnol. Oceanogr. 45: 871–880.

_____ and D. B. Chadwick. 2001. Assessing the dispersal and exchange of brachyuran larvae between regions of San Diego Bay, California and nearshore coastal habitats using elemental fingerprinting. J. Mar. Res. 59: 53–78.

Dufour, V., C. Pierre and J. Rancher. 1998. Stable isotopes in fish otoliths discriminate between lagoonal and oceanic residents of Taiaro Atoll (Tuamoto Archipelago, French Polynesia). Coral Reefs 17: 23–28.

Edmands, S., P. Moberg and R. S. Burton. 1996. Allozyme and mitochondrial DNA evidence of population subdivision in the purple sea urchin, *Strongylocentrotus purpuratus*. Mar. Biol. 126: 443–450.

Ennevor, B. C. 1993. Mass marking coho salmon, *Oncorhynchus kisutch*, fry with lanthanum and cerium. Fish. Bull. U.S. 92: 471–473.

_____ and R. M. Beames. 1993. Use of lanthanide elements to mass mark juvenile salmonids. Can. J. Fish. Aquat. Sci. 50: 1039–1044.

Fowler, J. A. 1970. Control of vertebrae number in teleosts- an embryological problem. Q. Rev. Biol. 45: 148–167.

Gaines, S. D. and M. D. Bertness. 1992. Dispersal of juveniles and variable recruitment in sessile marine species. Nature 360: 579–580.

Gibbs, P. D. L. and M. C. Schmale. 2000. GFP as a genetic marker scorable throughout the life cycle of transgenic zebra fish. Mar. Biotechnol. 2: 107–125.

Giles, M. A. and E. M. Attas. 1993. Rare earth elements as internal batch marks for rainbow trout: Retention, distribution and effects on growth of injected dysprosium, europium and samarium. Trans. Amer. Fish. Soc. 122: 289–297.

Gillanders, B. M. and M. J. Kingsford, M. J. 1996. Elements in otoliths may elucidate the contribution of estuarine recruitment to sustaining coast reef populations of a temperate reef fish. Mar. Ecol. Prog. Ser. 141: 13–20.

Grosberg, R. K. 1991. Sperm-mediated gene flow and the genetic structure of a population of the colonial ascidian *Botryllus schlosseri*. Evolution 45: 130–142.

Hansen, H. J. M. and A. T. A. Fattah. 1986. Long-term tagging of elvers, *Anguilla anguilla*, with radioactive europium. J. Fish Biol. 29: 535–540.

Hayes, J. L. 1991. Elemental marking of arthropod pests in agricultural systems: Single and multi-generational marking. Southwestern Entomological Suppl. 14: 37–47.

Hedric, P. W. 2001. Invasion of transgenes from salmon or other genetically modified organisms into natural populations. Can. J. Fish. Aquat. Sci. 58: 841–844.

Hettler, W. F. 1984. Marking otoliths by immersion of marine fish larvae in tetracycline. Trans. Amer. Fish. Soc. 113: 370–373.

Jones, G. P., M. J. Milicich, M. J. Emslie and C. Lunow. 1999. Self-recruitment in a coral reef fish population. Nature 402: 802–804.

Kennedy, B. D., C. L. Folt, J. D. Blum and C. P. Chamberlain. 1997. Natural isotope markers in salmon. Nature 387: 766–767.

Kennedy, B. P., J. D. Blum, C. L. Folt and K. H. Nislow. 2000. Using natural strontium isotopic signatures as fish markers: methodology and application. Can. J. Fish. Aquat. Sci. 57: 2280–2292.

Koehn, R. K., R. I. E. Newell and F. Immerman. 1980. Maintenance of an aminopeptidase allele frequency cline by natural selection. Proc. Nat'l. Acad. Sci. 77: 5385–5389.

Kordos, L. M. and R. S. Burton. 1993. Genetic differentiation of Texas Gulf coast population of the blue crab *Callinectes sapidus*. Mar. Biol. 117: 227–233.

Kristiansen, T. S., K. E. Jørstad, H. Otterå, O. I. Paulsen and T. Svåsand. 1997. Estimates of larval survival of cod by releases of genetically marked yolk-sac larvae. J. Fish Biol. 51: 264–283.

Lehtonen, H., K. Nyberg, P. J. Vuorinen and A. Leskela. 1992. Radioactive strontium (^{85}Sr) in marking whitefish [*Coregonus lavaretus* (L.)] larvae and the dispersal of larvae from river to sea. J. Fish Biol. 41: 417–423.

Levin, L. A. 1990. A review of methods for labeling and tracking marine invertebrate larvae. Ophelia 32: 115–144.

_____, D. Huggett, P. Myers, T. Bridges and J. Weaver. 1993. Rare-earth tagging methods for the study of larval dispersal by marine invertebrates. Limnol. Oceanogr. 38: 346–360.

Levy, J. A., L. F. Marins and A. Sanchez. 2000. Gene transfer technology in aquaculture. Hydrobiologia 420: 91–94.

Loosanoff, V. L. and H. C. Davis. 1947. Staining of oyster larvae as a method for studies of their movement and distribution. Science 106: 597–598.

Li, G. and D. Hedgecock. 1998. Genetic heterogeneity, detected by PCR-SSCP, among samples of larval Pacific oysters (*Crassostrea gigas*) supports the hypothesis of large variance in reproductive success. Can. J. Fish. Aquat. Sci. 55: 1025–1033.

Lu, J.-K., T. T. Chen, S. K. Allen, T. Matsubara and J. C. Burns. 1996. Production of transgenic dwarf surf clams, *Mulinia lateralis*, with pantropic retroviral vectors. Proc. Nat'l. Acad. Sci. 93: 3482–3486.

Moberg, P. E. and R. S. Burton. 2000. Genetic heterogeneity among recruit and adult red sea urchins, *Strongylocentrotus franciscanus*. Mar. Biol. 136: 773–784.

Mohler, J. W. 1997. Immersion of larval Atlantic salmon in calcein solutions to induce a non-lethally detectable mark. North Amer. J. Fish. Manag. 17: 751–756.

Moran, A. L. 2000. Calcein as a marker in experimental studies of newly hatched gastropods. Mar. Biol. 137: 893–898.

Muir, W. M. and R. D. Howard. 1999. Possible ecological risks of transgenic organism release when transgenes affect mating success: sexual selection and the Trojan gene hypothesis. Proc. Nat'l. Acad. Sci. 96: 13,853–13,856.

Murray, C. B. and T. D. Beacham. 1988. Responses of meristic characters in chum salmon (*Oncorhynchus keta*) to temperature changes during development. Can. J. Zool. 67: 596–600.

Nagiec, M., K. Dabrowski, C. Nagiec and E. Murawska. 1988. Mass-marking of coregonid larvae and fry by tetracycline tagging of otoliths. Aquaculture Fish Management 19: 171–178.

Olson R. R. and R. McPherson. 1987. Potential vs. realized larval dispersal: fish predation on larvae of the ascidian *Lissoclinum patella* (Gottschaldt). J. Exp. Mar. Biol. Ecol. 110: 245–256.

Perez-Enrique, R. and N. Tanigushi. 1999. Use of microsatellite DNA as genetic tags for the assessment of a stock enhancement program of red sea bream. Fish. Sci. 65: 374–379.

Pollard, M. J., M. J. Kingsford and S. C. Battaglene. 1999. Chemical marking of juvenile snapper, *Pagrus auratus* (Sparidae), by incorporation of strontium into dorsal spines. Fish. Bull. U.S. 97: 118–131.

Reinert, T. R., J. Wallin, M. C. Griffin, M. J. Conroy and M. J. Van Den Avyle. 1998. Long-term retention and detection of oxytetracycline marks applied to hatchery-reared larval striped base, *Morone saxatilis*. Can. J. Fish. Aquat. Sci. 55: 539–543.

Roughgarden, J., S. Gaines and H. Possingham. 1988. Recruitment dynamics in complex life cycles. Science 241: 1460–1466.

Rowley, R. J. and D.I. Mackinnon. 1995. Use of the fluorescent marker calcein in biomineralisation studies of brachiopods and other marine organisms. Bulletin de l'Institut Oceanographique (Monaco), SPEC. 14: 111–120.

Schroder, S. L., C. M. Knudsen and E. C. Volk. 1995. Marking salmon fry with strontium chloride solutions. Can. J. Fish. Aquat. Sci. 52: 1141–1149.

Seber, G. A. F. 1982. The estimation of animal abundance and related parameters, 2nd ed. MacMillan, New York.

Secor, D. H., M. G. White and J. M. Dean. 1991. Immersion marking of larval and juvenile hatchery-produced striped bass with oxytetracycline. Trans. Amer. Fish. Soc. 120: 261–266.

_____, E. D. Houde and D. M. Monteleone. 1995. A mark-release experiment on larval striped bass *Morone saxatilis* in a Chesapeake Bay tributary. ICES J. Mar. Sci. 52: 87–101.

Snyder, R. J., B. A. McKeown, K. Colbow and R. Brown. 1992. Use of dissolved strontium in scale marking of juvenile salmonids: Effects of concentration and exposure time. Can. J. Fish. Aq. Sci. 49: 780–782.

Swain, D. P. and C. C. Lindsey. 1985. Meristic variation in a clone of the cyprinodont fish *Rivulus marmoratus* related to temperature history of the parents and of the embryos. Can. J. Zool. 64: 1444–1455.

_____ and K. T. Frank. 2000. Depth-dependent vertebral number of Atlantic cod (*Gadus morhua*) on the Magdalen Shallows and Scotian Shelf: stock mixing or microgeographic trends within populations? Can. J. Fish. Aquat. Sci. 57: 2393–2401.

Swearer, S. E., J. E. Caselle, D. W. Lea and R. R. Warner. 1999. Larval retention and recruitment in an island population of a coral-reef fish. Nature 402: 799–802.

Thorrold, S. R., C. M. Jones, P. K. Swart and T. E. Targett. 1998. Accurate classification of nursery areas of juvenile weakfish (*Cynoscion regalis*) based on chemical signatures in otoliths. Mar. Ecol. Prog. Ser. 173: 253–265.

_____, C. Latkoczy, P. K. Swart and C. M. Jones. 2001. Natal homing in a marine fish metapopulation. Science 291: 297–299.

Tsukamoto, K. 1985. Mass-marking of ayu eggs and larvae by tetracycline-tagging of otoliths. Bull. Jpn. Soc. Sci. Fish. 51: 903–911.

_____. 1988. Otolith tagging of ayu embryo with fluorescent substances. Nippon Suisan Gakkaishi. 54: 1289–1295.

Twining, B. S., J. J. Gilbert and N. S. Fisher. 2000. Evidence of homing behavior in the coral reef mysid *Mysidium gracile*. Limnol. Oceanogr. 1845–1849.

Unkenholz, E. G., M. L. Brown and K. L. Pope. 1997. Oxytetracycline marking efficacy for yellow perch fingerlings and temporal assays of tissue residues. Progr. Fish-Culturist 59: 280–284.

Volk, E. C., S. L. Schroder and K. L. Fresh. 1990. Inducement of unique otolith banding patterns as a practical means to mass-mark juvenile pacific salmon. Amer. Fish. Soc. Symp. 7: 203–215.

_____, _____ and J. J. Grimm. 1999. Otolith thermal marking. Fish. Res. 43: 205–219.

Vuorinen, P. J., K. Nyberg and H. Lehtonen. 1998. Radioactive strontium (^{85}Sr) in marking newly hatched pike and success of stocking. J. Fish Biol. 52: 268–280.

Wilson, C. A., D. W. Beckman and J. M. Dean. 1987. Calcein as a fluorescent marker of otoliths of larval and juvenile fish. Trans. Amer. Fish. Soc. 116: 668–670.

Wilson R. R., K. A. Donaldson, M. E. Frischer and T. B. Young. 1997. Mitochondrial DNA control region of common snook and its prospect for use as a genetic tag. Trans. Amer. Fish. Soc. 126: 594–606.

Wilson, R. R. Jr. and K. A. Donaldson. 1998. Restriction digest of PCR-amplified mtDNA from fin clips is an assay for sequence genetic "tags" among hundreds of fish in wild populations. Mol. Mar. Biol. Biotechnol. 7: 39–47.

Zhu, Z., G. Li, L. He and S. Chen. 1985. Novel gene transfer into the fertilized eggs of gold fish (*Carassius auratus* L. 1758). Zeitsch. F. Angewandt Ichthyol. 1: 31–34.

Zilinskas, R. A. and P. J. Balint. 1998. Genetically engineered marine organisms: environmental and economic risks and benefits. Kluwer Academic, Boston, Massachusetts.

ADDRESSES: (S.R.T.) *Biology Department, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543.* (G.P.J.) *School of Marine Biology and Aquaculture, James Cook University, Townsville, 4811, Queensland, Australia.* (M.E.H.) *Department of Biological Sciences, 508 Life Sciences Building, Louisiana State University, Baton Rouge, Louisiana 70802.* (R.S.B.) *Marine Biology Research Division, Scripps Institution of Oceanography, University of California San Diego, La Jolla, California 92093-0202.* (S.E.S., R.R.W.) *Department of Ecology, Evolution, and Marine Biology, University of California, Santa Barbara, California 93106.* (J.E.N.) *Department of Biology, University of Louisiana, Lafayette, Louisiana 70504.* (S.G.M.) *Bodega Marine Laboratory, University of California, Westside Road, Bodega Bay, California 94923.* CORRESPONDING AUTHOR: (S.R.T.) *E-mail:* <*sthorrold@whoi.edu*>.