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Distribution and Identification of Fish Eggs in an Internal Wave Transport Mechanism

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Abstract

Internal waves have been proven to transport invertebrate larvae onshore, but there has been little indication on whether internal waves transport fish eggs. Fish eggs are typically buoyant and are often found in neustonic samples, and internal waves often cause fronts that transport oils and other light particles. This research aims to determine whether there are patterns to the distribution of fish eggs. One possibility is that offshore fish eggs can be transported onshore, to nearshore nursery habitats. Before 2003, when genetic barcoding was proposed as an identification mechanism, fish eggs could only be identified visually, using color, size, and shape. However, this method can be unreliable, so this research utilizes the COI barcoding gene to identify fish eggs to a species in samples taken in the South La Jolla State Marine Preserve, an area known to experience internal waves. Samples were taken within internal wave events and also at times without internal waves for control samples. Overall, 14 species of fish were found throughout the samples via DNA barcoding, 13 in event samples, and 7 in control samples. One sample was unable to be identified to a species, due to a higher level of species divergence from the most closely related sequenced specimen. No statistically significant differences were seen in abundance or distribution, although weak trends were seen that more fish eggs were found during event samples than in controls, and that more species were found in event samples. After calculation of diversity indices, only Simpson's evenness showed significant differences, with event samples being more evenly distributed than control samples. Overall, fish eggs were found during internal wave fronts, indicating that they can be transported. However, insights as to how or why these eggs are transported are unclear, including whether or not the eggs are simply carried along in the fronts by accident.

Introduction

Internal waves are a primary area of study in the ocean, because of the important role they play in mixing and turbulence. For one, the internal wave field is the only serious candidate for the contribution of enough energy for mixing to occur across densities (Venayagamoorthy & Fringer 2012). These internal waves can exist in any fluid that supports a density stratification (St Laurent et al. 2012), which typically occurs around the pycnocline in the ocean (da Silva et al. 2012). When the water column is well stratified in coastal waters, internal waves occur fairly frequently (Baines 1986). Multiple studies have verified the existence of internal waves off the coast of Southern California, including in San Diego County (Arthur 1955; Pineda 1995; Ufford 1947; Winant & Bratkovich 1981).

In addition, internal waves have been known to cause convergence of water at the surface, which creates surface slicks. These slicks are concentrations of small, buoyant particles (Ewing 1950). In addition, larval forms of fish and invertebrates can be highly concentrated in these slicks (Arthur 1955). However, there are times in which the slicks do not form. One reason for this was proposed by Shanks and Wright (1987). They found that winds greater than 7 m/s could cause the slicks to dissociate. Slicks are typically seen in the summer, as stratification is stronger during those months (Lennert-Cody & Franks 2002).

Since internal waves transport neustonic larvae, it is highly likely that fish eggs can also be transported in the surface slicks. Fish eggs are generally buoyant and transported in surface waters (Brewer et al. 1984; Motos & Coombs 2000). Fish eggs are largely buoyant because the fluid inside has less than half the osmotic concentration of seawater (Craik & Harvey 1987). However, there are many factors that affect the buoyancy of fish eggs. First, the salinity at egg generation is important. Fish eggs have a thick chorion, meaning that it is impermeable to water

once this chorion is in place, and that pre-ovulated eggs are permeable to water (Holliday 1969).

In addition, other behavioral factors have been researched as affecting buoyancy, including

spawning period (Chambers 1997), spawning time (Hiemstra 1962), and spawning depth

(Fletcher 1999). Finally, physical factors have also been implicated in affecting buoyancy. These

include light exposure (Mangor‐Jensen & Waiwood 1995) and turbulence (Sundby 1983).

In order to identify collected fish eggs to a species, this study makes use of DNA

barcoding technology. DNA barcoding, proposed in 2003 (Hebert et al. 2003), allows for the

identification of nearly any sample, even when morphological characteristics may be

indistinguishable. Hebert et al (2003) proposed the use of cytochrome c oxidase 1 (CO1) gene

for this barcoding property. This gene was suggested due to the fact that is possesses a high level

of diversity, but is also highly constrained. This gene is approximately 650 base pairs long,

meaning that it takes only a single cycle in a conventional sequencer (Hajibabaei et al. 2007).

Many studies have attempted to look at the effectiveness of the CO1 gene. For example, Ward et

al. (2005) analyzed the CO1 region of 207 species of Australian marine fish. They found that all

207 species could be differentiated, but some taxa showed more divergence in the sequence. One

reason the technology works for marine fishes is that only five percent of the genetic variance

seen between individuals comes from differences within a population (Ward et al. 1994).

Overall, this study seeks to address the question of whether or not fish eggs are

transported to nearshore habitats by internal waves, and whether there is a difference in diversity

based on the species found normally in La Jolla or those found in the internal wave samples. It is

predicted that internal wave samples have higher diversity, by transporting fish eggs from

offshore to nearshore, along with having higher abundance. Once fish eggs are collected,

identification through DNA barcoding will take place, and patterns of distribution based on

species will be analyzed. The null hypotheses are as follows: (1) there is no difference in abundance of fish eggs between control and event samples. (2) There is no difference in diversity in control and event samples. (3) Distribution of fish eggs within event samples is even. The findings suggest that fish eggs are transported in internal waves, and that internal wave samples may have higher species evenness than the fish eggs typically found in the region of study.

Materials and Methods

Sampling Method

This study takes place as a part of a three-year National Science Foundation grant awarded to Dr. Nathalie Reyns (University of San Diego, Environmental and Oceans Sciences Department), Jesus Pineda (Woods Hole Oceanographic Institute – WHOI), and Steven Lentz (WHOI). This grant (#1357290) is an analysis of the physical and biological processes involved in nearshore larval transport of invertebrates. Temperature monitoring equipment was set up off the coast of Southern California, in the South Marine State Preserve, at two sites. One site is located at a station depth of 2-4m, site A, the other at 8m depth, site B (Figure 1A). This sampling area is adjacent to a kelp forest (Figure 1B). Equipment was set up May to July 2014.
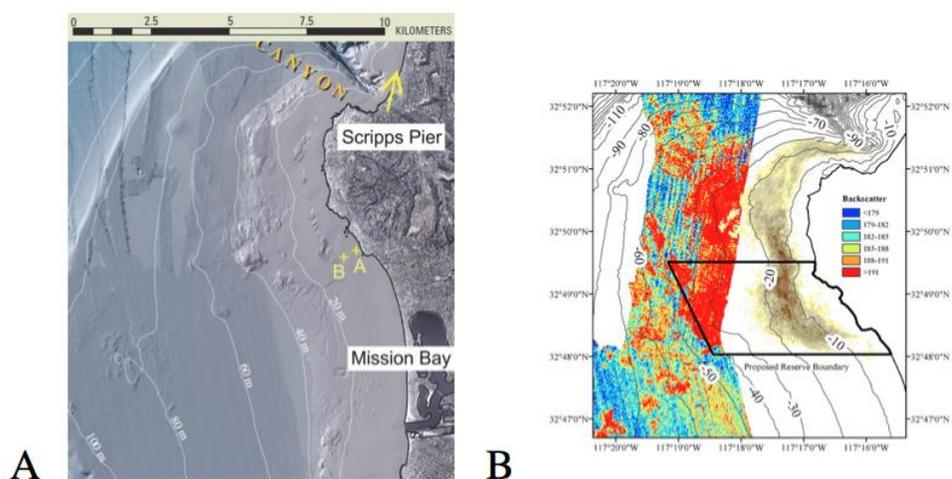

Figure 1: Area of study with (a) location of temperature equipment at a station depth of 2-4m (A) and 8m (B), and (b) the density of the kelp forest in the same region in gold.

When temperature oscillations signaling the arrival of an internal wave are read on the temperature monitoring equipment, samples were taken as an internal wave event. Control sampling occurred at times when internal wave events were not occurring. The general sampling methods were collected using Ebara semivortex pumps (300 liters/minute) with a 5cm diameter hose. Water pumped was filtered with a 106-μm mesh net. The water sampled was measured via a flow meter at every site. Once filtered, the samples were placed in 1-liter Nalgene bottles with 75% ethanol for transport and storage.

Event samples were composed of four sample times, before the internal wave front, during the front, and two samples taken after the front. The site depth for before and after samples was 4m, whereas the samples taken during the front were of variable site depth, moving with the internal wave front. At each sample time, samples were taken at four depths, the surface (0.15m), the bottom (0.2 MAB), and two intermediate depths (0.75m, 2.0m). Control samples were taken at set site depths, from 4m to 12m. At each site, samples were taken every 2m from the surface to the bottom. For this study, only site depths of 4m, 6m, and 8m were used in the control samples. In addition, control samples were taken on 5/23/14 and 6/11/14, and internal wave event samples were taken on 5/26/14, 6/3/14, and 6/4/14. One-way ANOVA statistical analysis and paired t-tests were performed on the abundances in control versus event samples, as well as between depth and sampling time in event samples.

Processing and DNA barcoding via CO1 method

After processing for invertebrate larvae, samples were processed for fish eggs. Ethanol from the samples was filtered out and stored, while the sample was put into very low concentrations of ethanol for sorting. Using a light microscope, samples were sorted for fish eggs. Fish eggs were placed into individual labeled 0.5 mL microcentrifuge tubes for later DNA

isolation. After all fish eggs from a particular sample were sorted, the ethanol was removed and the eggs rinsed with deionized water. The dry egg was crushed against the bottom of the tube and put into Tris-HCl, EDTA buffer, and stored at -20ºC.

Once DNA was isolated, PCR amplification occurred with forward (5'-TTCTCAACCAACCACAAAGACATTGG-3') and reverse (5'-TAGACTTCTGGGTGGCCAAAGCCTCA-3') CO1 primers. Verification of amplified DNA occurred using 1.5% agarose gel and SYBR® Safe DNA stain. The Quigen QIAquick® PCR purification kit was used to process successful PCR products for sequencing. Nanodrop analyses of the purified samples occurred and samples were then sent to Retrogen Inc. for sequencing. Once Sanger sequences were received, sequences were edited, trimmed and aligned using MEGA6 (Molecular Evolutionary Genetics Analysis Version 6.0), and then searched through NCBI's database via a nucleotide BLAST (blast.ncbi.nlm.nih.gov) used to identify eggs to the species level.

Phylogenetic analysis

Identifications were verified through the analysis of all samples, as well as 118 species commonly found in the Southern California region. Sequences were realigned and complied into a maximum likelihood tree using MEGA 6. Tree was analyzed using FigTree v1.4.2. Using a 2% divergence threshold, species were confirmed to species level. Pairwise difference was calculated in MEGA6 to verify species.

Diversity indices and statistical analysis

To measure biodiversity, several diversity indices were calculated. The Shannon-Wiener Diversity Index (H') was used to reflect evenness and species richness. In addition, Shannon's equitability (E_H) was calculated to focus more specifically on the evenness of individuals

between the species found during events or during control samples. Simpson's Evenness (E_D)

analyzes the uniformity of the ecosystem, to again look at evenness with respect to numbers of

organisms. Margalef's species richness (D) was calculated to analyze differences in species

richness between event and control samples. Finally, to measure the similarities between the

event samples and control samples, the Sorenson Similarity Index was calculated in matrix form.

One-way ANOVA analysis was performed on the first four diversity indices to determine

statistical differences. These tests were followed by analyses of abundance within internal wave

event samples, timing and depth calculations. An f-test followed by an unpaired t-test with equal

variance took place on abundance measures between control samples and event samples.

Results

Abundance of fish eggs in control and event samples

Overall, there was no significant difference

(P=0.182) between the control and event samples in

abundance of fish eggs per cubic meter. An f-test

(F=0.462), followed by a paired t-test with unequal

variances was performed. There was a general trend

that there were more fish eggs in the event samples (29

± 15) than in the control samples (9.5 ± 5) (Table 1).

Table 1: Number of fish eggs collected per cubic meter water. An f-test (P=0.462) followed by an unpaired t-test with equal variances (P=0.182) showed a weak trend.

Control Samples	
5/23/14	6
6/11/14	13
Average	9.5 ± 4.95
Event Samples	
5/26/14	16
6/3/14	45
6/4/14	26
Average	29 ± 14.73

Within an internal wave event, a one way ANOVA showed no statistically significant

differences in abundance per cubic meter between sampling times F(2,9) =0.9, P=0.440.

Generally, during (10 ± 3) and after (10 ± 13) showed higher values than before an internal wave

event (2 ± 2) (Figure 2). A one-way ANOVA between before and during samples showed

significant differences in abundance of fish eggs per meter cubed $F_{(1,5)} = 10.63$, $P=0.022$. In addition, no statistically significant differences were seen between depths that fish eggs were collected from $F_{(3,44)} = 1.07$, $P=0.372$ (Figure 3).
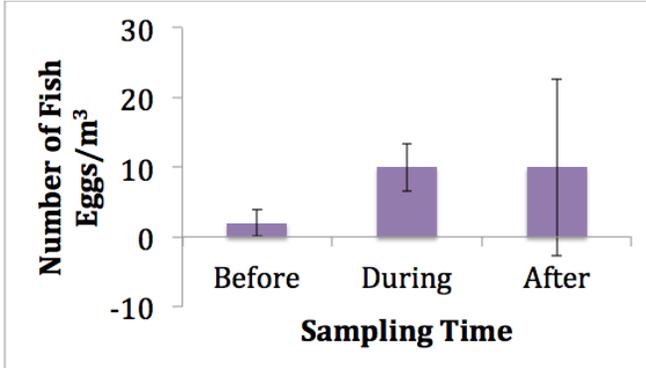

Figure 2: Average number of fish eggs found at each sampling time during internal wave events. Samples from all three events were normalized and averaged, with the standard deviation reflected in error bars.
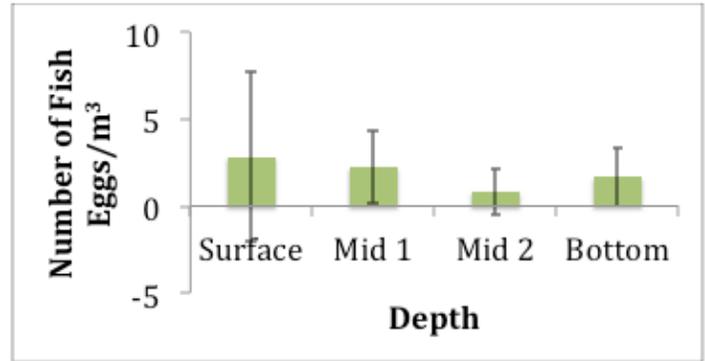
Figure 3: Average number of fish eggs found at each sampling depth during internal wave events. Samples from all three events were normalized and averaged, with the standard deviation reflected in error bars.

Generally, there were fewer fish eggs in the second intermediate depth, than the surface or bottom samples (Figure 3). The highest number of species during an internal wave event was found in the surface sample (Table 2), as well as the highest number of fish eggs identified.

Table 2: Identification of fish eggs found in event samples by depth via DNA barcoding of CO1. Total number of individuals is given for each depth.

Species	Surface	Mid 1 (0.75m)	Mid 2 (2.0m)	Bottom
Anisotremus davidsonii	1	0	0	1
Cheilotrema saturnum	1	0	1	1
Citharichthys stigmaeus	1	1	0	0
Cynoscion parvipinnis	1	1	2	0
Halichoeres semicinctus	3	2	1	2
Menticirrhus undulatus	0	2	0	0
Oxyjulis californica	7	9	2	3
Paralabrax clathratus	0	0	0	0
Roncador stearnsii	1	0	2	3
Scomber japonicus	3	1	0	0
Semicossyphus pulcher	0	0	1	1
Trachurus symmetricus	1	0	0	0
Umbrina roncador	1	0	0	0
Xenistius californiensis	7	6	1	3
Number of individuals	27	22	10	14
Number of species	11	7	7	7

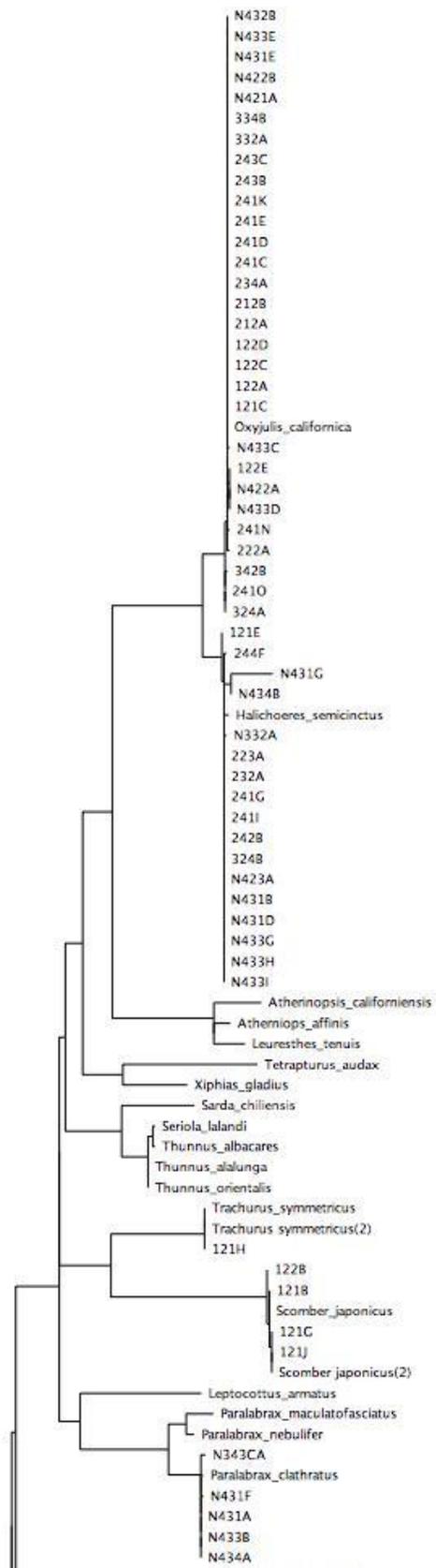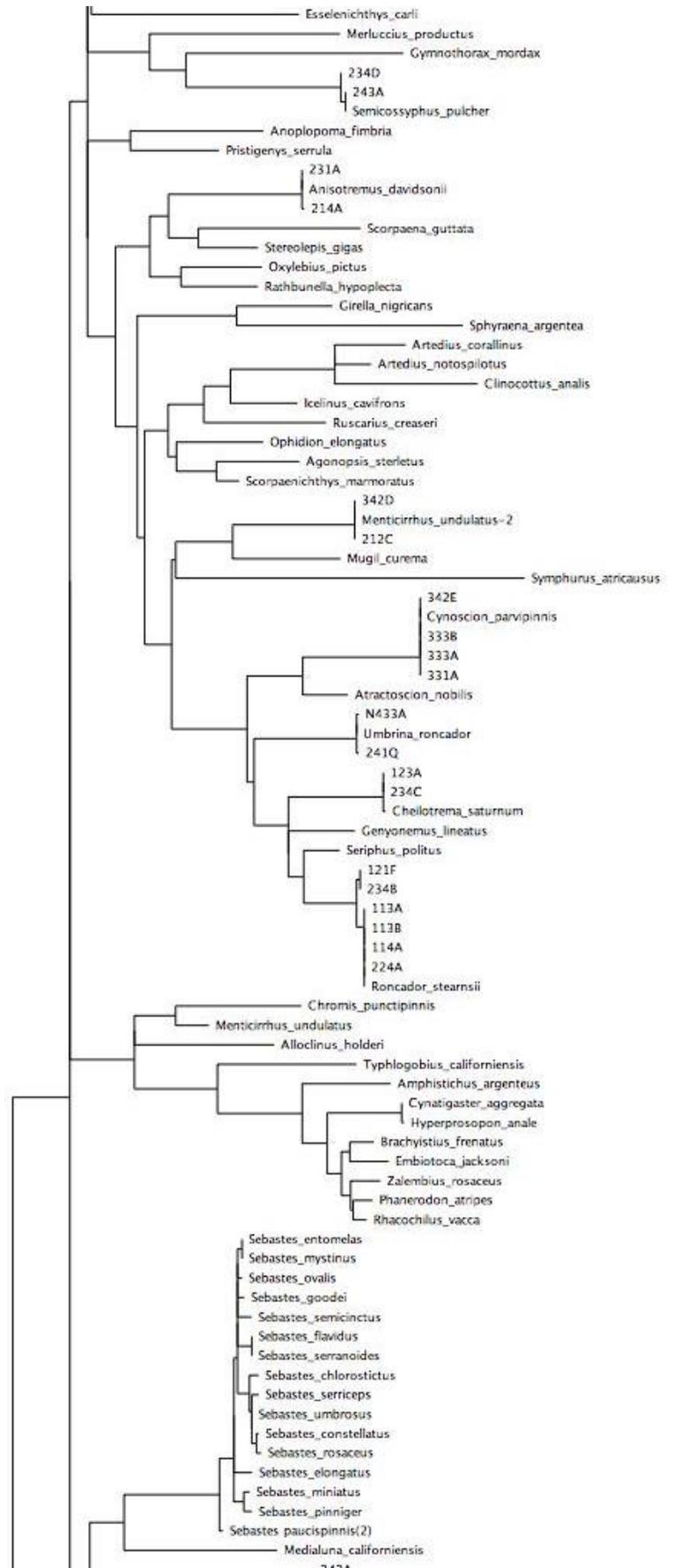*Identification of fish eggs and phylogenetic analysis*

     Identification via BLAST search yielded 14 different species, 13 found in event samples

and 7 found in control samples (Table 3). Identifications were considered to be true if percent

identification in the BLAST search was over 98%. On average, control samples showed a fewer

number of species (3.5 ± 2) than in event samples (7 ± 2) (Table 2). Via a paired t-test with equal

variance (F=0.911) there is no significant difference between these values (P=0.159); however, a

weak trend is confirmed. The most commonly found species included *H. semicinctus, O.*

californica, and *X. californiensis.* Three species, *O. californica*, *S. japonicus*, and *X.*

californiensis, were largely concentrated in the surface water, while *R. stearnsii* and *S. pulcher*

were concentrated at the bottom (Table 2).

Table 3: Identification of fish eggs via DNA barcoding of CO1. 73.7% of fish eggs found produced a successful sequence used for an NCBI nucleotide BLAST search for species identification.

Species	Control 1	Control 2	Event 1	Event 2	Event 3
Anisotremus davidsonii	0	0	0	2	0
Cheilotrema saturnum	0	0	1	1	1
Citharichthys stigmaeus	1	0	0	2	0
Cynoscion parvipinnis	0	0	0	0	4
Halichoeres semicinctus	1	6	1	6	1
Menticirrhus undulatus	0	0	0	1	1
Oxyjulis californica	0	6	5	12	4
Paralabrax clathratus	0	6	0	0	0
Roncador stearnsii	0	0	4	2	0
Scomber japonicas	0	0	4	0	0
Semicossyphus pulcher	0	0	0	2	0
Trachurus symmetricus	0	0	1	0	0
Umbrina roncador	0	1	0	1	0
Xenistius californiensis	0	2	0	16	1
Number of individuals	2	21	16	45	12
Number of species	2	5	6	10	6

     Phylogenetic analysis largely confirmed the results of the BLAST search. One sample,

N431G showed a high level of divergence from *Halichoeres semicinctus*, likely indicating a

specimen that has yet to be sequenced (Figure 4). Pairwise difference between species identified

as *H. semicinctus* and N431G was 0.074 for all calculations.

N432B
N433E
N431E
N422B
N421A
334B
332A
243C
243B
241K
241E
241D
241C
234A
212B
212A
122D
122C
122A
121C
Oxyjulis_californica
N433C
122E
N422A
N433D
241N
222A
342B
241O
324A
121E
244F
N431G
N434B
Halichoeres_semicinctus
N332A
223A
232A
241G
241I
242B
324B
N423A
N431B
N431D
N433G
N433H
N433I
Atherinopsis_californiensis
Atherniops_affinis
Leuresthes_tenuis
Tetrapturus_audax
Xiphias_gladius
Sarda_chiliensis
Seriola_lalandi
Thunnus_albacares
Thunnus_alalunga
Thunnus_orientalis
Trachurus_symmetricus
Trachurus_symmetricus(2)
121H
122B
121B
Scomber_japonicus
121G
121J
Scomber japonicus(2)
Leptocottus_armatus
Paralabrax_maculatofasciatus
Paralabrax_nebulifer
N343CA
Paralabrax_clathratus
N431F
N431A
N433B
N434A

Esselenichthys_carli
Merluccius_productus
Gymnothorax_mordax
234D
243A
Semicossyphus_pulcher
Anoplopoma_fimbria
Pristigenys_serrula
231A
Anisotremus_davidsonii
214A
Scorpaena_guttata
Stereolepis_gigas
Oxylebius_pictus
Rathbunella_hypoplecta
Girella_nigricans
Sphyraena_argentea
Artedius_corallinus
Artedius_notospilotus
Clinocottus_analis
Icelinus_cavifrons
Ruscarius_creaseri
Ophidion_elongatus
Agonopsis_sterletus
Scorpaenichthys_marmoratus
342D
Menticirrhus_undulatus-2
212C
Mugil_curema
Symphurus_atricausus
342E
Cynoscion_parvipinnis
333B
333A
331A
Atractoscion_nobilis
N433A
Umbrina_roncador
241Q
123A
234C
Cheilotrema_saturnum
Genyonemus_lineatus
Seriphus_politus
121F
234B
113A
113B
114A
224A
Roncador_stearnsii
Chromis_punctipinnis
Menticirrhus_undulatus
Alloclinus_holderi
Typhlogobius_californiensis
Amphistichus_argenteus
Cynatigaster_aggregata
Hyperprosopon_anale
Brachyistius_frenatus
Embiotoca_jacksoni
Zalembius_rosaceus
Phanerodon_atripes
Rhacochilus_vacca
Sebastes_entomelas
Sebastes_mystinus
Sebastes_ovalis
Sebastes_goodei
Sebastes_semicinctus
Sebastes_flavidus
Sebastes_serranoides
Sebastes_chlorostictus
Sebastes_serriceps
Sebastes_umbrosus
Sebastes_constellatus
Sebastes_rosaceus
Sebastes_elongatus
Sebastes_miniatus
Sebastes_pinniger
Sebastes paucispinnis(2)
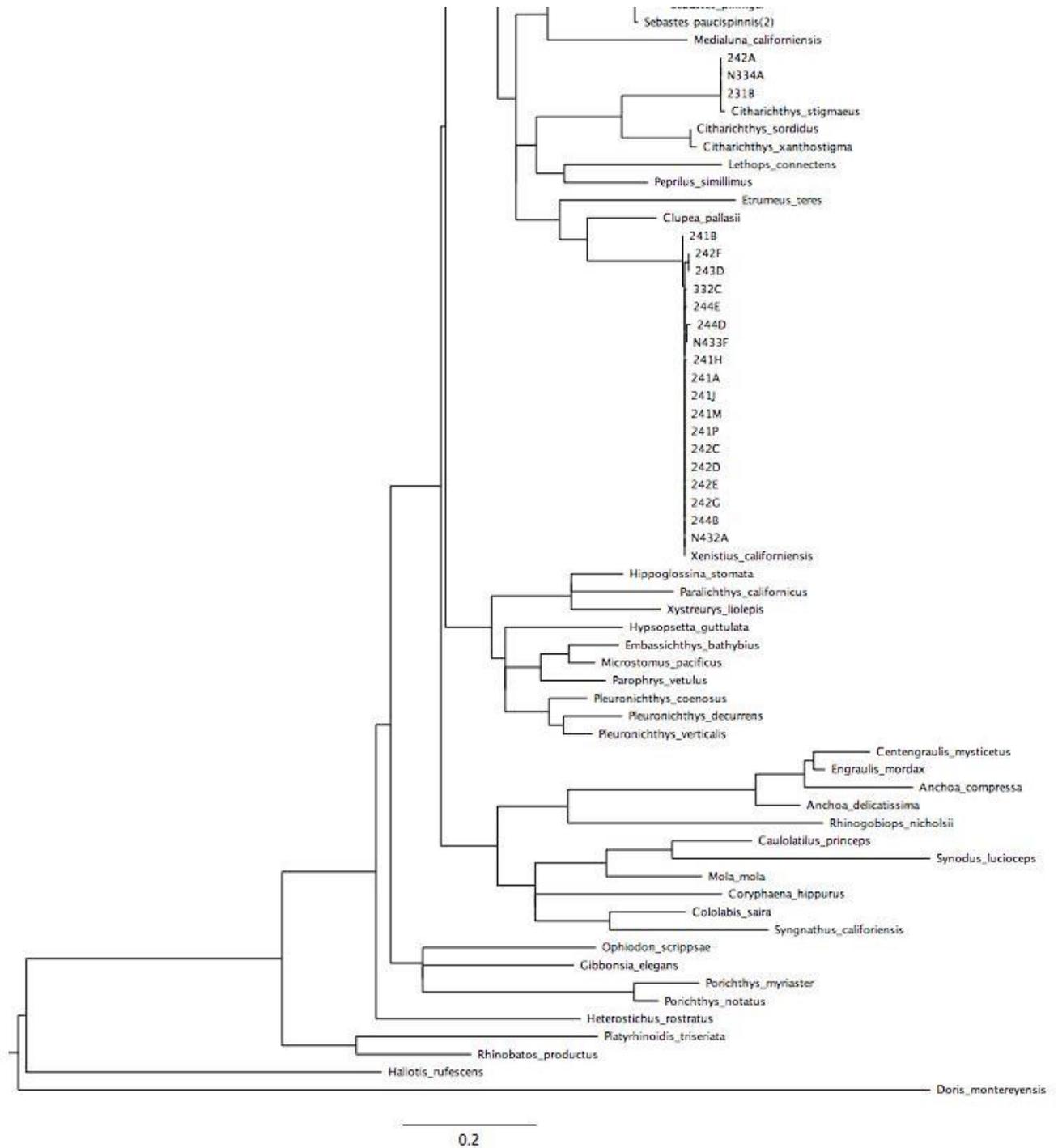Medialuna_californiensis
242A

Figure 4: Maximum likelihood tree from CO1 sequences. Tree was constructed using all event and control samples with sequences longer than 196 base pairs. In addition, CO1118 species from the San Diego region were included in the tree, and aligned with the samples.

Diversity Indices

No significant difference was seen in Shannon-Wiener Diversity (P=0.222), Margalef's

species richness (P=0.447), Shannon's Equitability indices (P=0.507) via a one-way ANOVA

(Table 4). However, Simpson's Evenness showed statistically significant differences between the

event samples and the control samples in a one-way ANOVA $F_{(3,14)}=4.8$, P=0.017, with the

event samples being more even (Table 4). In addition, there were significant differences between

the control samples and before the front (P=0.019), and the control samples and during the front

(P=0.013). Control samples and after the front showed a weak trend of after the front showing

more evenness (P=0.092).

Table 4: Diversity indices based on sampling time in internal wave events. One-way ANOVA was calculated for each diversity index.

Diversity Index	Before	During	After	Control	P-value
Shannon-Wiener (H')	0.53±0.62	1.39±0.64	0.73±0.67	0.45±0.57	0.222
Margalef's Richness (D)	1.93±0.93	2.94±0.63	2.18±0.5	2.89±0.96	0.447
Shannon's Equitability (E_H)	0.49±0.56	0.95±0.09	0.53±0.49	0.45±0.49	0.507
Simpson's Evenness (E_D)	0.72±0.48	0.87±0.22	0.46±0.46	0.06±0.1	0.017*

Simpson's evenness comparisons ranged from 0.4 to 0.67, generally. The most

differences were between the first intermediate depth and the bottom. The highest amount of

similarity was seen between the second intermediate depth and the bottom samples. Comparing

the sampling times in events, before samples showed the most differences with the after and

control samples, whereas during samples were the most different from the control samples

(Table 5,6).

Table 5: Sorensen's similarity index between depths during events.

	Surface	Mid 1	Mid 2	Bottom
Surface	-	-	-	-
Mid 1	0.67	-	-	-
Mid 2	0.67	0.57	-	-
Bottom	0.67	0.43	0.85	-

Table 6: Sorensen's similarity index between events and control samples.

	Before	During	After	Control
Before	-	-	-	-
During	0.57	-	-	-
After	0.43	0.55	-	-
Control	0.36	0.40	0.67	-

Discussion

Abundance and identification of fish eggs

Overall, a general trend was seen that more fish eggs were found during and after fronts in event samples than before the fronts, and also that more fish eggs were found in event samples than in control samples. These are all values that are expected if fish eggs are transported by internal waves. However, this is simply a weak trend, so conclusions cannot be directly drawn from this data. Since fish eggs were in the internal wave samples, it can be concluded that fish eggs can be congregated in fronts, similar to what occurs with other neustonic larvae (Jillet & Zeldis 1985; Shanks 1988; Shanks & Wright 1987). This larva includes larval fish, barnacles, and crabs, all of which can weakly swim.

Fish eggs were found throughout the water column, with the lowest number found at 2.0m depth. Fish eggs are generally buoyant, and although many factors affect this, one of the main factors is the spawning depth. The majority of fish species that were identified in this 2.0m depth were pelagic spawning fish, with two species spawning near the bottom, *C. stigamaeus* and *C. saturnum*. There is little information about the spawning behavior of he majority of the species and the buoyancy of their fish eggs. The species that spawn near the bottom did not follow that pattern, but were instead found near the surface or throughout the water column. Since these samples were taken at an average depth of 4.0m, the eggs could have experienced mixing due to breaking waves in the surf zone. If samples were taken from further away from shore, the eggs may have been found at their typical spawned depth.

Overall, all fish identified have been known to spawn between late spring and summer (FishBase). The species concentrated in the surface waters, *O. californica*, *S. japonicus*, and *X. californiensis*, are all pelagic spawners with eggs that float free in the upper layers (Froese &

Pauly 2015). Only one species identified, with one individual, is a true offshore species. *T. symmetricus* is a pelagic-oceanic fish that typically spawns anywhere from 8 to 240 miles offshore, whereas adults school up to 500 miles away from the coast. On the other hand, 8 of the 14 species found, including the three most common, are known to live in the kelp forest, and thus, the front collected eggs that were already located in the area of the study.

One sample, N431G did not match any sequences in GenBank, indicating a sample from a species not yet sequenced, or a high level of divergence within the species. However, multiple other samples matched *H. semicinctus* within 2% divergence, indicating the first possibility. A search for congeners of *H. semicinctus* produced no results for the Southern California area. With climate change occurring, the waters off the coast of Southern California are getting warmer, due to a change in wind patterns (Johnstone & Mantua 2014). Changes in sea temperature, as caused by climate change, were studied by Perry et al. (2005), and they found that in the North Sea, two-thirds of species shifted latitude or depth within 25 years. Therefore, it is possible that the egg came from a species that is not typically found in the area, but with climate change, may be shifting habitats.

Analyzing the diversity indices, Shannon-Wiener, Margalef's richness, and Shannon's equitability all take into account species richness, whereas Simpson's evenness only looks at species evenness. Thus, it is possible that species richness between control and event samples is similar, whereas species evenness is more even during event samples, in the nearshore region of La Jolla. The samples reflected moderate levels of similarity via Sorensen's similarity index. The samples with the highest levels of difference included 2.0m versus 0.2MAB, and the surface samples to everything else, as well as the control and after samples. In terms of depth, samples were taken getting closer to the surf zone, which has a high rate of mixing due to surface waves

interacting with the bottom topography. As a result, it is difficult to draw conclusions from this data. However, it does appear as though there is little correlation between the species found at different depths, as the surface sample is very similar to the bottom sample.

Nursery habitats

The hypothesis that offshore fish eggs are transported onshore in order to reach safer habitats comes into question, since the majority of the species found in the samples are species typically found in the kelp forest. As a result, the juveniles are already using the area as a nursery habitat and do not need to be transported from offshore. Since some of the species found are not most commonly found in the kelp forest, the hypothesis could certainly still be valid, although more investigation is required. For one, multiple sample sites, including contrasts between the open coast and the kelp forest should be considered, along with samples from near other nursery habitats in the San Diego Region. Second, some of the fish eggs that were from offshore species may have already reached the kelp forest and gotten hung up within the dense kelp. Therefore, samples should be taken on the edge of the kelp forest furthest away from shore, to determine whether or not more offshore species are found in internal wave samples.

General improvements

In this study, there are experimental improvements that should occur for further studies. For one, the statistical analysis done in this paper is constrained due to the number of samples taken. Since only two event samples and three normal samples were considered, it is hard to draw statistically sound conclusions from the data. In fact, some trends may resolve themselves with more samples, and outliers in the data can be identified and taken out of the statistical analysis to more adequately analyze the results. Second, the samples in this study were taken specifically sampling for invertebrate larvae. These larvae are much smaller than fish eggs, and

when sampling for fish eggs, different methods should be employed. Fish eggs are typically collected using plankton nets, as they catch large amounts of fish eggs at once (Taggart & Leggett 1984). However, these are used when dragged from a boat over a long period of time, which is not feasible for this study. Smaller hose size, as used in this study, causes different drag in the water than larger hoses, and will thus collect smaller items that are unable to avoid this drag, although drag is felt from further away with larger hoses or nets.

In addition, fish eggs were stored in ethanol after collection. In the samples that remained in ethanol for longer periods of time before processing, there was a lower percent yield of successful sequences. Expedient processing of the fish eggs found could have a higher percentage yield, due to less risk of DNA damage due to ethanol. Another possibility would be to use a higher percentage of ethanol, since in this study, 75% ethanol was used.

Conclusions

In conclusion, fish eggs can be caught up in fronts of internal waves, since they were found in the internal wave samples. Generally, more fish eggs were found in the event samples than in the control samples, along with more species being generally found in these samples. Species evenness showed significant differences between event samples and control samples, whereas species richness did not. However, few offshore species were found, and the majority of the species are commonly found in the kelp forest. In order to more adequately gauge whether offshore species use internal waves to transport fish eggs onshore would require more extensive sampling methods, most specifically sampling on the offshore side of the kelp forest.

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