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Location and transport of early life stages of West Australian Dhufish *Glaucosoma hebraicum*

Project No 2011/016

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1. NON TECHNICAL SUMMARY

2011/016 Location and transport of early life stages of West Australian Dhufish *Glaucosoma hebraicum*

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OBJECTIVES:

1) Finding early life stages of West Australian Dhufish *Glaucosoma hebraicum*
2) Unravelling transport areas of eggs and larvae and correlating them with physical and biological processes
3) Predicting larval sources and sinks and relating them to currents, salinity, temperature, chlorophyll and food

NON TECHNICAL SUMMARY:

OUTCOMES ACHIEVED TO DATE

A combination of methods have been developed and tested to locate for the first time early life stages (eggs and larvae) of West Australian Dhufish *Glaucosoma hebraicum* and provide a detailed map of their distribution at two sites off south-western Australia.

The project demonstrated that:

- The use of a hydrodynamic model can optimise and guide oceanographic sampling when there is a paucity of knowledge on spawning locations.
- The shipboard detection of larvae allows adaptive sampling maximising the chances of success.
- A properly interpreted particle (larvae) tracking model can throw light on early life history of *G. hebraicum*.
- There is high variability in the currents along the continental shelf between Cape Leeuwin and Cape Naturaliste (Western Australia), with clear implications for larval transport of West Australian Dhufish and other species important to commercial, recreational and charter fishers.
- There is variability in food supply for fish larvae in the Capes region that has consequences for larval survival.

The project developed:

- A species-specific PCR assay to detect *G. hebraicum* DNA from mixed zooplankton samples allowing near real time processing of plankton samples.
- A proof of concept for use of quantitative PCR analysis to establish an index of the biomass of West Australian Dhufish eggs and larvae in real time.

G. hebraicum is endemic to Western Australia and it occurs between the Recherche Archipelago near Esperance (31º51'S, 121º53'E), to Shark Bay (25°30′S 113°30′E / 25.5°S 113.5°E). Spawning occurs from November to April with the peak period in January and February. The suspected spawning areas are shallow reef outcrops and weed-covered sandy substrates. *G. hebraicum* is one of the most popular targets for recreational, charter and commercial fishers with fishing mortality exceeding natural mortality in recent assessments. *G. hebraicum* is slow growing and long-lived, potentially reaching over 40 y of age and populations of such long lived individuals are sustained by infrequent episodes of good recruitment. The stocks in recent years have been dominated by cohorts from a few strong year classes. Understanding the physical and biological processes that underpin recruitment success in *G. hebraicum* is of key importance to making predictions about the future productivity of the fishery. *G. hebraicum* larvae have never been found in the wild and are known only from the aquaculture trials.

The present study used an integration of hydrodynamic modelling, field sampling and rapid DNA-based identification to provide the first detailed location map of early life stages *G. hebraicum*. The results of the particle tracking model showed that larvae in nearshore habitats, in 20 to 30 m deep waters, are rapidly carried offshore unless they vertically migrate and then use onshore return flows to reverse the seaward transport. The hydrodynamic model of the Capes region, in south-western Australia, indicated that larvae are most likely to be retained around Capes Naturaliste (33º31'S, 115º00'E) and Leeuwin (34º, 22'S, 115º08'E) and in Geographe Bay (33º35'S, 115º15'E).

Satellite drifters deployed during the study in 20 and 40 m depth in various locations between Cape Naturalist and Cape Leeuwin in January and February 2012 showed high current variability in space and time with consequent implications for egg and larval transport. The time and place of spawning, in particular, variations in the locations of spawning across the continental shelf relative to the offshore southerly-flowing Leeuwin and inshore northerly –flowing Capes currents will play a crucial role in larval retention and recruitment or offshore dispersal and loss from the population. The Capes Current was evident most of the time in January and February 2012 but drifter data showed an absence of the current occasionally. The current speeds were also very variable occasionally exceeding 1 knot. Such peak water movements are estimated to be able to transport eggs and larvae ~ 50 km in a 24 hour period.

DNA analysis based on quantitative PCR provided an index of *G. hebraicum* eggs and larvae biomass. This index was significantly positively correlated with temperature and negatively correlated with salinity but there was no statistically significant association with other environmental variables including nutrients or chlorophyll biomass.

This project has provided a proof of concept of the methods employed. A combination of hydrodynamic modelling, field sampling and rapid genetic identification provided baseline data on the location of eggs and larvae of *G. hebraicum* in the Capes region of south-western Australia. This is the first step towards developing research into larval recruitment of this species. In particular, the project has demonstrated the advantage of using DNA techniques for identification of target taxa from mixed zooplankton samples to adapt sampling and maximise success rate. Furthermore, the quantitative PCR method tested here can be used as a relative index of biomass. This may be able to be developed to provide some form of recruitment index for WA *G. hebraicum* and thus as an early indicator of the expected future strength of individual cohorts as they recruit to the fishery.

The methods developed in this project can be applied to similar situations when there is little knowledge of how organisms are distributed or are difficult to identify. Examples include measurement of recruitment, monitoring the impacts of climate change on shifts of organisms or biosecurity.

KEYWORDS: Fishery management, recruitment, eggs, larvae, West Australian Dhufish, *Glaucosoma hebraicum*, hydrodynamic modelling, particle tracking, genetics, Western Australia.

2. Acknowledgments

The project was jointly funded by the Fisheries Research and Development Corporation (FRDC project 2011/016 Location and transport of early life stages of West Australian Dhufish (*Glaucosoma hebraicum*), the Government of Western Australia Department of Fisheries and the CSIRO Sustainable Ocean Ecosystems and Living Resources research theme. The following people provided advice during the project: Gary Jackson, Francisco (Pancho) Neira, and Jim Greenwood. Steve Guy was instrumental in originally setting up the drifter programme within WAMSI 1. We thank Brett Crisafulli (Department of Fisheries Western Australia) and Michelle Gardner (Murdoch University), for specimens used in comparative DNA testing. We gratefully acknowledge the skills of the masters and crew of R.V. Naturaliste and R.V. Linnaeus.

3. BACKGROUND

Our proposal was developed in consultation with staff from the Department of Fisheries WA Divisions of Research and Aquatic Management and its research scientists have been closely involved in the design of the project. *G. hebraicum* is a key indicator species for the demersal fish in south west of WA. Its biology has been studied only relatively recently by Hesp et al (2002). His study identified insufficient knowledge of larval biology, ecology and settlement of West Australian Dhufish *Glaucosoma hebraicum*. This species is a slow growing and long-lived with individuals reaching over 40 years (Hesp et al. 2002). It has been recognised that populations of such long-lived species are sustained by infrequent episodes of good recruitment, termed the storage effect (Warner and Chesson, 1985). Storage effect is also recognised as a mechanism of species recovery for example in case of Chesapeake Bay striped Bass where egg production by spawners older than 15 years was a key element of the rapid recovery (Secor, 2007). Catches of *G. hebraicum* in the commercial and recreational sectors of the West Coast Demersal Scalefish Fishery in recent years have been dominated by cohorts produced during four relatively strong consecutive years of recruitment from 1993 to 1997 (Wise et al. 2007, FRR 163 and FRR 174) suggesting that storage effect is also an important mechanism in the biology of this species. *G. hebraicum* is likely to have multiple nursery habitats adjacent to adult habitats and multiple sources of recruits (Fairclough et al. 2013). Larval transport is likely to be extensive, gene flow is extensive and larval behaviour potentially has a significant influence on extent of transport (Berry et al, 2012). This present project bridges the gap in understanding the ecology of early life stages of *G. hebraicum* by locating its eggs and larvae and relates their presence to oceanographic conditions, and environmental variables. The timeline of this project is one year to provide proof of concept and test of the methods. The project provides baseline data that is essential for long term studies and longer term studies will be required to effectively address inter-annual variability in egg and larval abundance and location in relation to environmental variables. The extension of the project will provide information on biology of species to management which could be used to

develop monitoring programs to aid our interpretation of recruitment strength. This could pro-actively inform management of future strong/weak recruitment to the fishery.

In this report we present a combination of methods including hydrodynamic modelling and genetics that allowed us to adjust our oceanographic sampling on a daily basis resulting in the first detailed map of the locations of *G. hebraicum* larvae at two sites in south-western Australia. We describe larval transport based on the results of modelling and observations from drifters. We also relate temperature, salinity, nutrients and abundance of phytoplankton to the abundance of larvae. The relationship between physics, biology and larvae is described for the Capes site since it was area of highly intensive surveys encompassing six weeks of daily sampling over three months.

4. NEED

G hebraicum is an iconic species and is a commercially and recreationally important finfish in Western Australia. Together with snapper (*Pagrus auratus*) it accounts for almost half of the commercial catch of the West Coast Demersal Scalefish Interim Managed Fishery (WCDSIMF) which operates along the west coast of Australia between 26°30'S and 115°30'E and is one of the most popular fish for recreational anglers. In recent years, commercial and recreational fishers reported that they moved further offshore to catch *G. hebraicum* suggesting that heavy fishing in shallower, inshore demersal waters (20 - 250 m depths) in the WC Bioregion had reduced their stock abundance. In addition to greater fishing effort, improvements in technology e.g. GPS has increased fishing efficiency. Stock depletion was also indicated in the stock assessments in 2005-2006 and 2007-2008 (Mitsopoulos and Molony, 2010). Another concern is the decrease in the proportion of *G. hebraicum* > 13 years of age in the catches from 28 to 9% over the last decade. The limited knowledge of this species' larval biology points to very limited dispersal over its range enhancing concerns about the vulnerability of *G. hebraicum* to localised overexploitation. Understanding the physical and biological processes that underpin recruitment success in *G. hebraicum* is of key importance in forecasting future productivity and management of the stock and the WCDSF. Recent research indicated that the recruitment strength will depend on spawning output of adults, oceanographic conditions and food availability during the planktonic stage. Lack of knowledge of the location and transport mechanisms of eggs and larvae fundamentally limits understanding of the recruitment of *G. hebraicum*. This project aims at locating the early life stages and their transport areas such that sustainability for this species can be pursued.

5. OBJECTIVES

1) Finding early life stages of West Australian Dhufish *Glaucosoma hebraicum*
2) Unravelling transport areas of eggs and larvae and correlating them with physical and biological processes
3) Predicting larval sources and sinks and relating them to currents, salinity, temperature, chlorophyll and food

6. METHODS

Field sampling

Locations and time of sampling

Sampling sites were chosen in consultation with scientists from the Western Australian Department of Fisheries based on *G. hebraicum* catches and anecdotal reports of *G. hebraicum* spawning areas from fishers (Mackie et al, 2009). Most of the sampling was undertaken in the Capes area from Cape Naturaliste to Cape Leeuwin (Figure 1). An additional site north-east of Rottnest Island was selected based on reports of sightings of juveniles from the NRM funded project (Mitsopoulos and Molony, 2010) and sites trawled during 2009 (Photo 1).

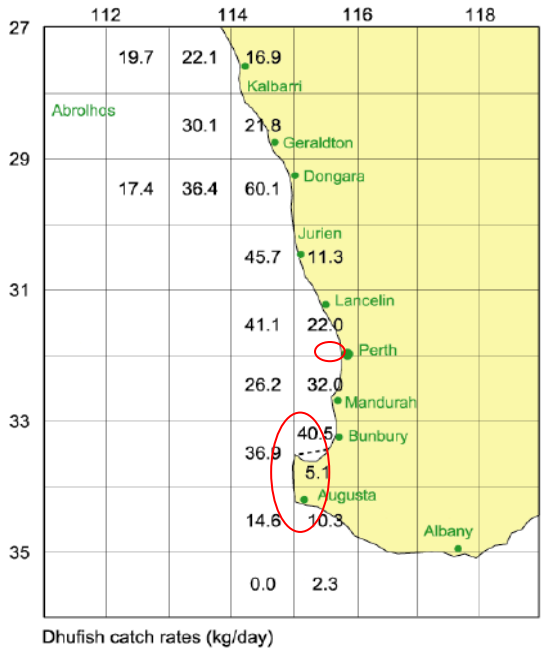


Figure 1 Map of south-western Australia showing approximate sampling locations (circled in red) and G. *hebraicum* catch rates in 60 nm blocks (Fisheries Occasional Publication No. 34). Please note that the map represents historical catch rates.

Photo 1 Image from towed video showing juvenile *G. hebraicum* (photo courtesy Mr Brad Adams, Department of Fisheries, WA) in habitat N-E of Rottnest Island.

G. hebraicum is a multiple spawner spawning in the vicinity of limestone or coral reefs from November to April with peak spawning time in late January and early February (Hesp et al 2002). We sampled from 6 to 18 December 2011, 16 to 30 January and 14 to 28 February 2012 in the Capes area and 14 to 15 March and 12 to 13 April 2012 in the Perth metro area. Appendix 1 includes summary information of all the stations.

Temperature, Salinity and Fluorescence (CTD data)

Temperature, salinity and fluorescence profiles were obtained in real time by lowering SBE 19plus profiler with two auxiliary photosynthetically available radiation (PAR) and fluorometer sensors. Data was captured using CSIRO CTD acquisition software, CAP which outputs the data directly to the netCDF scan files. Data was processed using custom Matlab scripts that follow standard processing techniques outlined in the manufacturers' documentation (http://www.seabird.com/pdf_documents/datasheets/SoftwareBrochure4PageDec09.pdf).

We used data acquired from the downcast. Salinity was calibrated against bottle salinity collected at the bottom depth at every second station. The northward wind stress from http://aodaac2-cbr.act.csiro.au:8080/opendap/imos/GHRSST/L3P/ABOM-L3P_GHRSST-SSTsubskin-AVHRR_MOSAIC_01km/ was used to define the strength of the Capes Current.

Nutrients

Water samples for nutrients were taken from Niskin's bottles triggered at surface, 10 m, 25 m and bottom depth with one of the depths substituted to sample at the chlorophyll maximum. All nutrients except ammonium were analysed using a flow injection analysis while ammonium concentration was determined using a flow injection analysis, gas diffusion, derivatisation - fluorescence detection method. For dissolved silica, Si species were reacted with molybdate at 45ºC pH 1.2 and then reduced with stannous chloride to form a heteropoly blue complex which was measured at 820 nm (Wolters, 2003). Phosphate was reacted with molybdate and antimony

potassium tartrate in acid medium which was then reduced with ascorbic acid to form a blue complex which was measured at 880 nm (Diamond, 1988). Nitrate was first reduced to nitrite by passing it through a copperised cadmium column. Nitrite was then reacted with sulfanilamide under acidic conditions to form a diazonium salt which was then coupled with N-(1-naphthyl) ethylenediamine dihydrogenchloride to form a pink complex which was measured at 520 nm (Diamond, 1999). For ammonium a sodium hydroxide solution was added to liberate ammonia which was then diffused across a PTFE membrane (HPMSD) to react with ortho-phthaldialdehyde (OPA) - sulfite solution to form a fluorescent derivative. This solution was then measured with an excitation wavelength of 310 nm and an emission wavelength of 390 nm (Watson, 2005). The detection limits are 0.05 µM for nitrates, phosphates and silica and 0.02 µM for ammonium.

Phytoplankton

Chlorophyll *a* was used as a measure of phytoplankton biomass. Water for chlorophyll *a* was collected with Niskin bottles from the surface and chlorophyll maximum layers. The chlorophyll maximum layer was determined from the fluorescence profile observed in real time during CTD casts. Water samples were filtered under low vacuum (<100 mm Hg) onto 25 mm diameter glass fibre filters (Whatman GF/FTM; nominal mesh size = 0.7 µ) (total phytoplankton) and 5 µm NitexTM mesh (large phytoplankton). Pigments were extracted overnight in 90% acetone in the dark at 4 ºC and measured using a Turner Designs model TD 700TM fluorometer which had been previously calibrated with pure Chl *a* (Sigma Chemical Co.) and chlorophyll *a* was calculated following standard methods (Parsons et al., 1984). Small phytoplankton biomass was obtained by subtracting large fraction biomass from total phytoplankton.

Zooplankton and Ichthyoplankton

Zooplankton was sampled with 355 and 100 µm, 50 cm diameter Bongo nets towed obliquely from the depth of the station to the surface during the day. Bongo nets were equipped with flowmeters to measure the volume of water filtered. In December and part of January we also sampled surface water using a neuston net (1m^2, 500 µm mesh) at night. This sampling was discontinued in favour of maximising day sampling with Bongo nets because of the low presence of eggs and larvae of *G. hebraicum* in the neuston compared with the Bongo nets. Each plankton sample was split using a Folsom plankton splitter and one part was preserved in 5% buffered formalin and the other part frozen in -20 ºC for genetic analysis.

Drifters

Two drifters were deployed in the Capes Region during the second survey cruise in January, and 8 others were released during the February survey:

Cruise period: 16 to 30 January 2012

Drifter (Ref.)	Release date	Nominal release position
#6670 (Ref1)	18/01/2012	-33° 36.4'S / 114° 58.3'E
#8060 (Ref4)	19/01/2012	-33° 31.4'S / 115° 03.6'E

Cruise period: 14 to 28 February 2012

Drifter (Ref.)	Release date	Nominal release position
#9660 (FF1)	15/2/2012	-33° 27.0'S / 115° 13.1'E
#0660 (FF2)	15/2/2012	-33° 29.8'S / 115° 00.1'E
#2670 (Ref2)	16/2/2012	-33° 42.1'S / 114° 46.5'E
#1690 (Ref3)	16/2/2012	-33° 40.5'S / 114° 55.5'E
#1650 (FF3)	17/2/2012	-34° 04.7'S / 114° 53.8'E
#5440 (FF4)	17/2/2012	-34° 07.2'S / 114° 48.7'E
#7660 (FF5)	20/2/2012	-34° 25.8'S / 115° 03.6'E
#3440 (FF6)	20/2/2012	-34° 26.3'S / 115° 00.2'E

All the drifters were provided by the Department of Fisheries as an "in-kind" contribution to the project. They were produced by the Canadian company MetOcean Data Systems using a design developed for the World Ocean Circulation Experiment (WOCE). Each drifter consists of a spherical surface float containing the electronics and a surface temperature sensor, with a subsurface "holey-sock" drogue 6.4 m long tethered at a nominal depth of 15 m below the water surface, thus covering the depth layer 12 to 18 m below the surface. All the drifters were programmed for transmission (via the Iridium satellite system) of hourly temperatures and 3-hourly positions.

The 3-hourly positions and hourly temperatures were received and stored on a secure website by Joubeh Technologies, from which the data were periodically downloaded on demand at the Department of Fisheries for processing and analysis. The positional fixes were checked for duplicate records (which were deleted) and for missing positions (single gaps were linearly interpolated, but longer gaps were left unfilled), and the trajectories plotted – each midnight fix was assigned a larger symbol to represent the daily progress of the drifter and to facilitate comparison

with the modelled daily current vectors. The 3-hourly drift speeds and directions were calculated and then plotted as a time-series in conjunction with the surface temperatures.

In addition to the position fixes and water temperatures, the tension in the cable connecting the drogue to the surface float was monitored as an indication of whether the drogue was still attached to the cable. It appears that none of the drogues became detached during the two ship survey periods, although some of the drifters seem to have subsequently lost their drogues.

For practical reasons, the drifters were not recovered at the end of each cruise and so have continued following the currents. Some have subsequently either run aground on the coast or have ceased operating, while the others have dispersed widely north, south and offshore of their release points. Only those portions of the trajectories during the 2-week cruises are discussed here.

Hydrodynamic Model

A high resolution hydrodynamic model was used to simulate the coastal currents along the Western Australian coast and passive particles were seeded in the model domain to simulate the dispersal of *G. hebraicum* larvae and eggs following water movement. The hydrodynamic model, configured with the Regional Ocean Modelling System (ROMS), covers the western coast of Australia from 21°S to 35°S and 108°W to 116°W with a high resolution of about 2-km in the coastal region and then gradually decreasing towards the deep ocean. There are 30 vertical levels in all water columns with fine resolutions in the top 100 m depth. The ROMS model was developed in the WAMSI and CSIRO-IMOS projects, and is capable of capturing key seasonal features of the Leeuwin Current and Capes Current, as validated against IMOS shelf mooring data. In this project the ROMS model is downscaled from the Bureau of Meteorology's operational ocean prediction system OceanMAPS and forced by OceanMAPS and is thus able to simulate and forecast shelf circulation along the Western Australia coast in near real time.

The particle tracking is modelled online with the hydrodynamic simulation from ROMS model. Trajectories of passive particles from designated release locations were simulated before the field surveys of eggs and larvae were undertaken. In each run of particle tracking simulation, the particles were initially released about 400m apart along the 20m isobath off the Capes region from 34.6°S to 32.4°S. The particles were released at two levels, at the surface and bottom layers of the model, every hour in a 5-hour time window from 7pm to 12pm local time, 1 week and 2 weeks prior to the field survey date.

In this project, larval behaviour such as vertical migration or temperature-dependent growth rates were not included in the model run because they are no known. However, we used the dispersal patterns 2-4 days after their initial release to assess the spatial aggregations, with the assumption that after a few days of their spawning and following hatching of fertilised eggs, larvae will migrate

vertically ion behaviour to maintain their horizontal location prior to settlement to demersal habitats. This would allow them to avoid the offshore surface drift due to the dominantly southerly wind.

Genetic detection of G. hebraicum from mixed zooplankton samples

Assay design

We designed a species-specific PCR assay to detect *G. hebraicum* DNA from mixed zooplankton samples. The test consisted of a species-specific primer pair that would amplify a 126 nucleotide (nt) fragment of the cytochrome oxidase subunit 1 mtDNA gene in the presence of *G. hebraicum* DNA only, and a primer pair that would amplify a 255 nt fragment of the 16s rRNA mtDNA gene in the presence of any bony fish DNA. This second DNA fragment functioned as a reaction control.

The *G. hebraicum*-specific primer was designed from an alignment of 34 sequences obtained from the international DNA database GenBank (www.ncbi.nlm.nih.gov/genbank), and from the FishBOL fish barcoding initiative (B. Ward, CSIRO, pers. comm,). These sequences included 17 species or close relatives to species that occur in south-western Australian coastal waters and were considered likely to spawn during the period of sampling (D. Fairclough, Western Australian Department of Fisheries pers. comm. table 1). Between 8 and 15 nucleotide mismatches were present between the primers and non-target species (mean 12.7). The universal primer pair was designed from an alignment of 12 16s sequences that included representatives of fishes considered likely to spawn in the region during the period of the study.

Family and Common Name	**Species name**	**Fish with ripe ovaries**	**peak spawning period**
Berycidae			
Bight Redfish	*Centroberyx gerrardi*	Jan-Apr	Jan-Apr
Glaucosomatidae			
West Australian Dhufish	*Glaucosoma hebraicum*	Oct-May	Dec-Mar
Labridae			
Baldchin Groper	*Choerodon rubescens*	Jul-Jan	Sept-Jan
Eastern Blue Groper	*Achoerodus_viridis*	Jun-Oct	Jul - Oct
Red Pigfish	*Bodianus_unimaculatus*	winter and spring	winter and spring

Lethrinidae			
Spangled Emperor	*Lethrinus nebulosus*	Oct-May	Nov-Mar
Redthroat Emperor	*Lethrinus miniatus*	Likely spring /summer	Data not available yet
Lutjanidae			
Red emperor	*Lutjanus sebae*	Jan-Dec	Oct-Mar
Percichthyidae			
Hapuku	*Polyprion oxygeneois*	May-Sept	Jun-Aug
Bass groper	*Polyprion americanus*	winter	winter
Platycephalidae			
Bar-tailed flathead	*Platycephalus endrachtensis*	Oct-May	Nov-Mar
Sciaenidae			
Mulloway	*Argyrosomus japonicus*	Oct-May	Nov-Jan
Serranidae			
Common Coral Trout	*Plectropomus leopardus*	Dec-Feb	Dec-Feb
Blacktip Grouper	*Epinephelus fasciatus*	summer in South Africa	
Sparidae			
Tarwhine	*Rhabdosargus sarba*	May-Dec	winter/spring
Silver seabream	Chrysophrys_auratus	Nov-Dec	Dec
Carangidae			
Silver Trevally	*Pseudocaranx wrighti.*	Jul-Dec	Sept-Dec

Table 1 Seventeen species or close relatives to species that occur in south-western Australian coastal waters and were considered likely to spawn during the period of sampling used to design the *G. hebraicum*-specific primer

These fragment were co-amplified in a single reaction as follows: 1 x PCR Buffer (Bioline, London, U.K.), 0.25 mM $MgCl_2$, 10 mM dNTPs, 0.8 mg/mL bovine serum albumin, 0.5 U Taq (MangoTaq, Bioline), primers (Table 2), and 1 µL DNA extract. Cycling conditions were as follows: 94ºC 2v minutes, (94ºC 30seconds, 58ºC 20s, 72ºC 30s) x 35, 72ºC 1 minute. Reactions were conducted on an Eppendorf EPS thermocycler (Eppendorf, Hilden, Germany). Fragments were visualised on 2.5% TBE agarose gels pre-stained with 0.25 x Gel-Red (Biotium) run at 90V for 30 minutes. All reactions included no template controls.

Primer type	Primer name	Primer sequence (5'-3')	PCR conc. (µM)
Dhufish-specific	Ghebcox1_540F	AACTCCTCTGTTCGTATGGGCGGTA	0.4
	Ghebcox1_666R	AAAGAATGGGGTCCCCTCCTCCGGAT	0.4
Universal	Fish16s_1160F	ACGAGAAGACCCTATGGAG	0.6
	Fish16s_1415R	CTGTTATCCCTAGGGTAAC	0.6

Table 2 Primers employed to detect *G. hebraicum* DNA from mixed zooplankton samples.

Primer testing

Both the species-specific and universal primers were tested across a range of annealing temperatures to determine the temperature that amplified both strongly. The relative amplification efficiency of each primer pair was adjusted with primer concentrations (Table 2). The test was applied to 20 *G. hebraicum* samples collected from throughout the species' range in Western Australia to ensure its ability to amplify. In addition it was tested against local non-target fish species, and the allopatric *Glaucosoma* congeners, *G. scapulare, G. magnificum, and G. buergeri* (Table 3). DNA was extracted from all fish specimens with a Qiagen Dneasy kit according to the manufacturers' recommendations.

Species	***Number of samples***	***Number of universal amplification***	***Number of G. hebraicum species-specific amplifications***
G. hebraicum	***20***	***20***	***20***
G. scapulare	***1***	***1***	***0***
G. magnificum	***5***	***5***	***0***
G. buergeri	***5***	***5***	***0***

Pagrus auratus	***10***	***10***	***0***
Choerodon rubescens	***10***	***10***	***0***
Platycephalus endrachtensis	***10***	***10***	***0***
Argyrosomus japonicus	***10***	***10***	***0***
Sillago bassensis	***10***	***9***	***0***
Sillago vittata	***10***	***10***	***0***
Sillago maculata	***10***	***10***	***0***
Sillago schomburgkii	***10***	***10***	***0***
Pseudocaranx georgianus	***10***	***10***	***0***
Othos dentex	***10***	***10***	***0***
Nemadactylus valenciennesi	***10***	***10***	***0***
Epinephelides armatus	***9***	***9***	***0***
Bodianus frenchii	***9***	***9***	***0***
Platycephalus laevigatus	***3***	***3***	***0***
Platycephasluis longispinis	***3***	***3***	***0***
Leviprora inospis	***3***	***3***	***0***

Table 3 Summary of species-specificity trials

The sequence of the 101 bp fragment obtained from our plankton samples using the *G. hebraicum* specific PCR was queried against major international DNA sequence databases (BOLD, www.boldsystems.org; and GenBank, http://blast.ncbi.nlm.nih.gov). These databases are repositories for DNA sequences collected from organisms throughout the world and include *Glaucosoma* sequences derived from the CSIRO National Fish Collection and Biodiversity Institute of Ontario. Both queries returned a 100% or near 100% match for *G. hebraicum*, and ≤ 90% matches to other species (Table 4).

Taxonomic Level	Taxon Assignment	Placement probability (%)
Phylum	Chordata	100
Class	Actinopterygii	100
Order	Perciformes	100
Family	Glaucosomatidae	100
Genus	Glaucosoma	100
Species	Glaucosoma hebraicum	100
A species level match has been made. This identification is solid unless there is a very closely allied congeneric species that has not yet been analyzed. Such cases are rare.		

Phylum	Class	Order	Family	Genus	Species	Specimen Similarity (%)
Chordata	Actinopterygii	Perciformes	Glaucosomatidae	Glaucosoma	*hebraicum*	100
Chordata	Actinopterygii	Perciformes	Glaucosomatidae	Glaucosoma	*hebraicum*	100
Chordata	Actinopterygii	Perciformes	Glaucosomatidae	Glaucosoma	*hebraicum*	100
Chordata	Actinopterygii	Perciformes	Glaucosomatidae	Glaucosoma	*hebraicum*	100
Chordata	Actinopterygii	Perciformes	Glaucosomatidae	Glaucosoma	*hebraicum*	100
Chordata	Actinopterygii	Perciformes	Glaucosomatidae	Glaucosoma	*hebraicum*	98.99
Chordata	Actinopterygii	Perciformes	Latridae	Latris	*lineata*	90.91
Chordata	Actinopterygii	Perciformes	Latridae	Latris	*lineata*	90.91
Chordata	Actinopterygii	Perciformes	Latridae	Latris	*lineata*	90.91
Chordata	Actinopterygii	Perciformes	Latridae	Latris	*lineata*	90.91
Chordata	Actinopterygii	Perciformes	Latridae	Latris	*lineata*	90.91
Chordata	Actinopterygii	Perciformes	Latridae	Latris	*lineata*	90.91
Chordata	Actinopterygii	Perciformes	Latridae	Latris	*lineata*	90.91
Chordata	Actinopterygii	Perciformes	Latridae	Latris	*lineata*	89.9
Chordata	Actinopterygii	Perciformes	Latridae	Latris	*lineata*	89.9
Chordata	Actinopterygii	Perciformes	Latridae	Mendosoma	*lineatum*	89.9
Chordata	Actinopterygii	Perciformes	Latridae	Mendosoma	*lineatum*	89.9
Chordata	Actinopterygii	Perciformes	Latridae	Mendosoma	*lineatum*	89.9
Chordata	Actinopterygii	Perciformes	Latridae	Mendosoma	*lineatum*	89.9
Chordata	Actinopterygii	Perciformes	Latridae	Mendosoma	*lineatum*	89.9

Table 4 Output from the Barcode of Life Database (BOLD) query.

Zooplankton processing.

Plankton samples for DNA analysis ranged between approximately 20 and 50 mL. Samples collected during January and February were processed directly on board the research vessel. Samples collected at other times were frozen at -20ºC and processed in the laboratory. Samples were thoroughly mixed before 15 mL was loaded into a disposable Ultra Turrax tube (model DT-20 or BMT-20, IKA Staufen, Germany) and vortexed at full speed for 60 seconds to completely homogenise the sample. Two hundred microlitres of plankton homogenate was taken with a wide bore 1000 µL tip and pipetted into a 1.5mL tube, which was centrifuged at 14,000 rpm for 60 seconds. The supernatant was removed and DNA was extracted from the remaining pellet processed according to standard Qiagen Dneasy kit protocol for animal tissues, but with the addition of 40 µL of proteinase K. DNA was eluted into 200 µL AE buffer.

A total of 334 samples were processed (DNA extraction and PCR test) on board the R.V. Naturaliste during January and February 2012. A further 100 samples collected during December 2011 and March-April 2012 were processed in the laboratory (Table 5). PCR reactions typically were conducted with groups of c. 20 samples. All reactions included a positive and negative control and amplified fragments were sized with a 100 bp ladder (Axygen).

Time	Location	Bongo 355	Bongo 100	neuston	Total
December	Capes	50	50		100
January	Capes	110	31	57	198
February	Capes	136			136
March-April	Perth Metro	40			40

Table 5 Summary of samples processed with DNA analysis in 2011-2012.

Verification of rapid PCR test field results.

We attempted to obtain DNA sequences from a subset of the samples that provided positive amplifications of the *G. hebraicum* specific PCR product during field analysis. To do so we conducted PCRs on these samples with the *G. hebraicum* specific primers only. PCR products were prepared for sequencing with BigDye Chemistry (Applied Biosystems, California, USA), and sequenced in forward and reverse on an ABI 3730 DNA sequencer. Sequences were checked by eye and edited with Geneious software (Biomatters, Auckland, New Zealand). Six of the 38 sequencing reactions did not provide readable sequence. The remainder were aligned to a dataset consisting of 23 fish species from the region that reproduce during our sampling period, three *Glaucosoma* congeners, and two *G. hebraicum* reference cox1 sequences (GenBank

accession: AB495192.1, EF609357.1) (Table 6). Alignments were conducted using the Muscle algorithm. Phylogenetic analysis was used to test the hypothesis that the observed plankton sequences differed significantly from the reference *G. hebraicum* sequences. This was conducted using neighbour-joining tree based on Tamura-Nei distances and 500 bootstrap replicates implemented in the Geneious software.

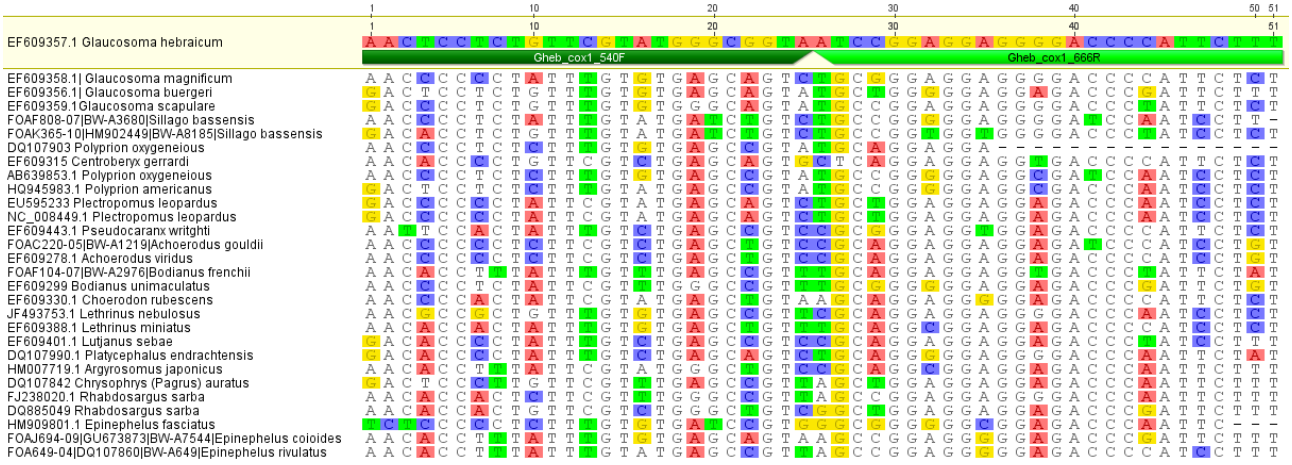


Table 6 Alignment of cytochrome oxidase sequences from non-target fishes corresponding to binding sites of *G. hebraicum*-specific primers (positions indicated by green arrows). Mismatches between *G. hebraicum* and non-target species are indicated by coloured boxes around nucleotides. GenBank accession numbers or FishBOL reference numbers (B. Ward, CSIRO, pers. comm.) precede species names. Sequence between primer binding sites has been omitted.

Sensitivity of the rapid PCR test

We conducted experiments to evaluate the sensitivity of the rapid PCR test. This involved spiking *G. hebraicum* fin tissue of known size into plankton samples (n = 16) that had previously given a negative result for the presence of *G. hebraicum* DNA. Because *G. hebraicum* larvae and eggs were not available, we selected tissue sizes that encompassed the dimensions of early (0-3 days; 2.05-2.43 mm) to late (35-45 days; 5.35-8.13 mm) larvae as described from aquaculture rearing studies (Pironet and Neira, 1998). Spiked plankton samples were processed through homogenisation, extraction and PCR according to the standard methodology. Two independent DNA extractions were conducted on each spiked plankton sample.

Quantitative analysis of plankton samples

As a proof of concept experiment we used quantitative PCR to establish the concentration of *G. hebraicum* DNA present in each of 90 plankton samples that provided positive results with the conventional PCR test. Reactions were conducted as per the *G. hebraicum* test except that the universal primers were omitted, the 1 x EVA Green intercalating dye (Biotium) was included, and the reaction was cycled 40 times. A five point standard curve in triplicate was used to establish concentrations. The concentration of *G. hebraicum* DNA in the spiked plankton samples was also

determined to provide indications of the biomass associated with DNA concentrations observed in the plankton. We used this biomass index to test for association with environmental variables.

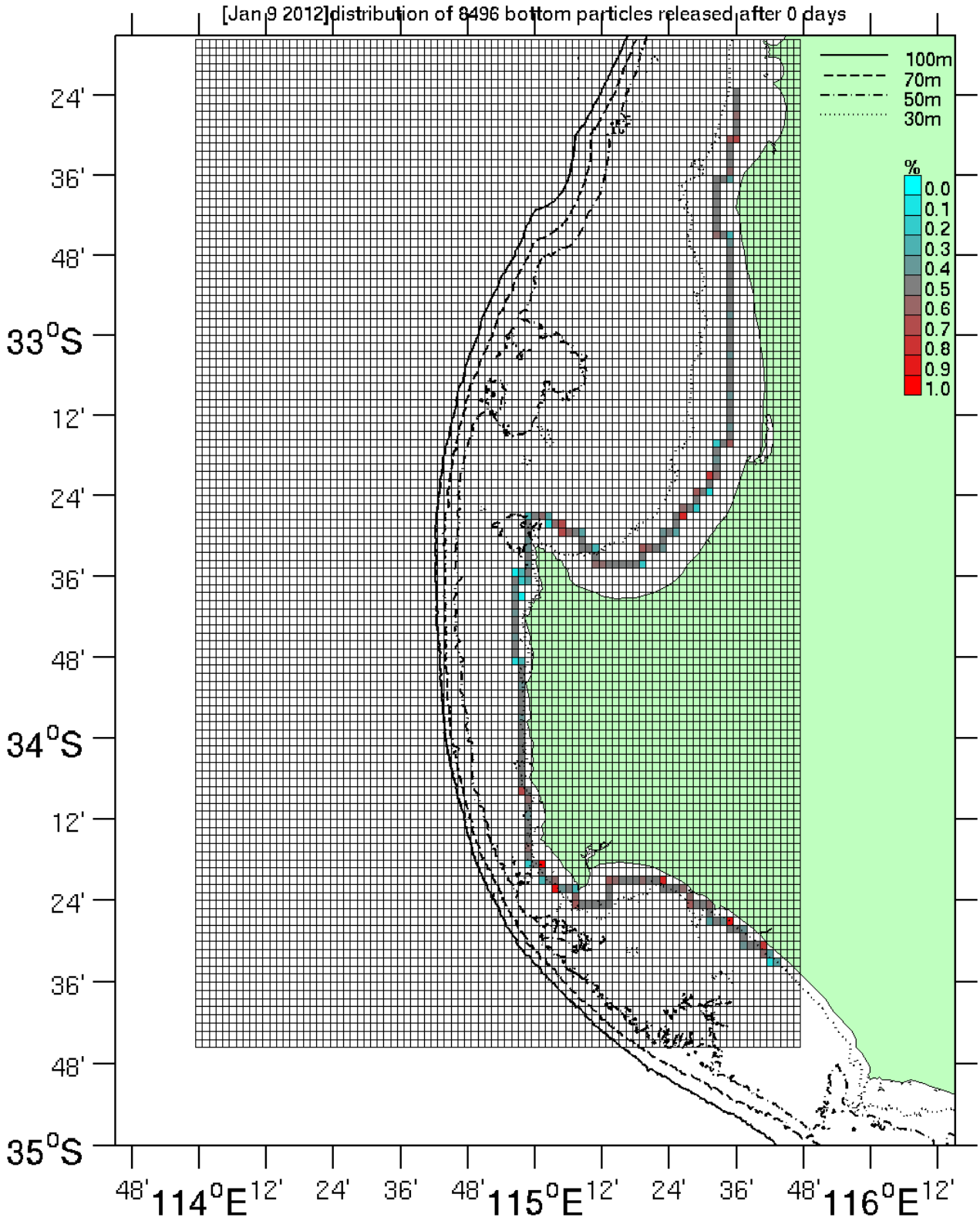
7. RESULTS/DISCUSSION

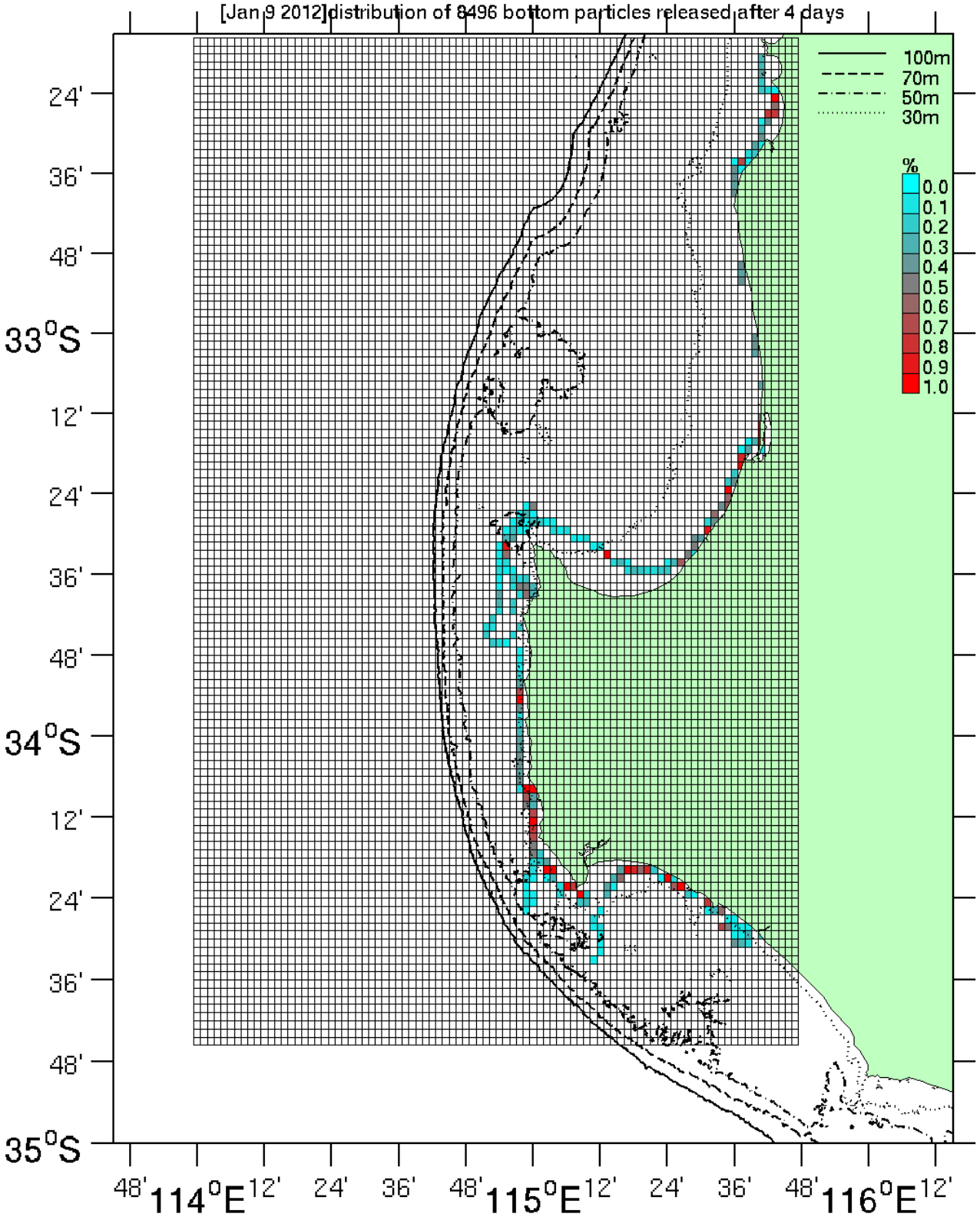
Objective:

1) Finding early life stages

Sampling strategy

We used a particle tracking model and drifters to decide on the location of our sampling. Figure 2 and Figure 3 show examples of model outputs that were used to guide our sampling.


[Jan 9 2012]distribution of 8496 bottom particles released after 0 days
100m
70m
50m
30m
%
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
24'
36'
48'
33°S
12'
24'
36'
48'
34°S
12'
24'
36'
48'
35°S
48'
114°E
12'
24'
36'
48'
115°E
12'
24'
36'
48'
116°E
12'


[Jan 9 2012]distribution of 8496 bottom particles released after 4 days
100m
70m
50m
30m
%
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
24'
36'
48'
33°S
12'
24'
36'
48'
34°S
12'
24'
36'
48'
35°S
48'
114°E
12'
24'
36'
48'
115°E
12'
24'
36'
48'
116°E
12'

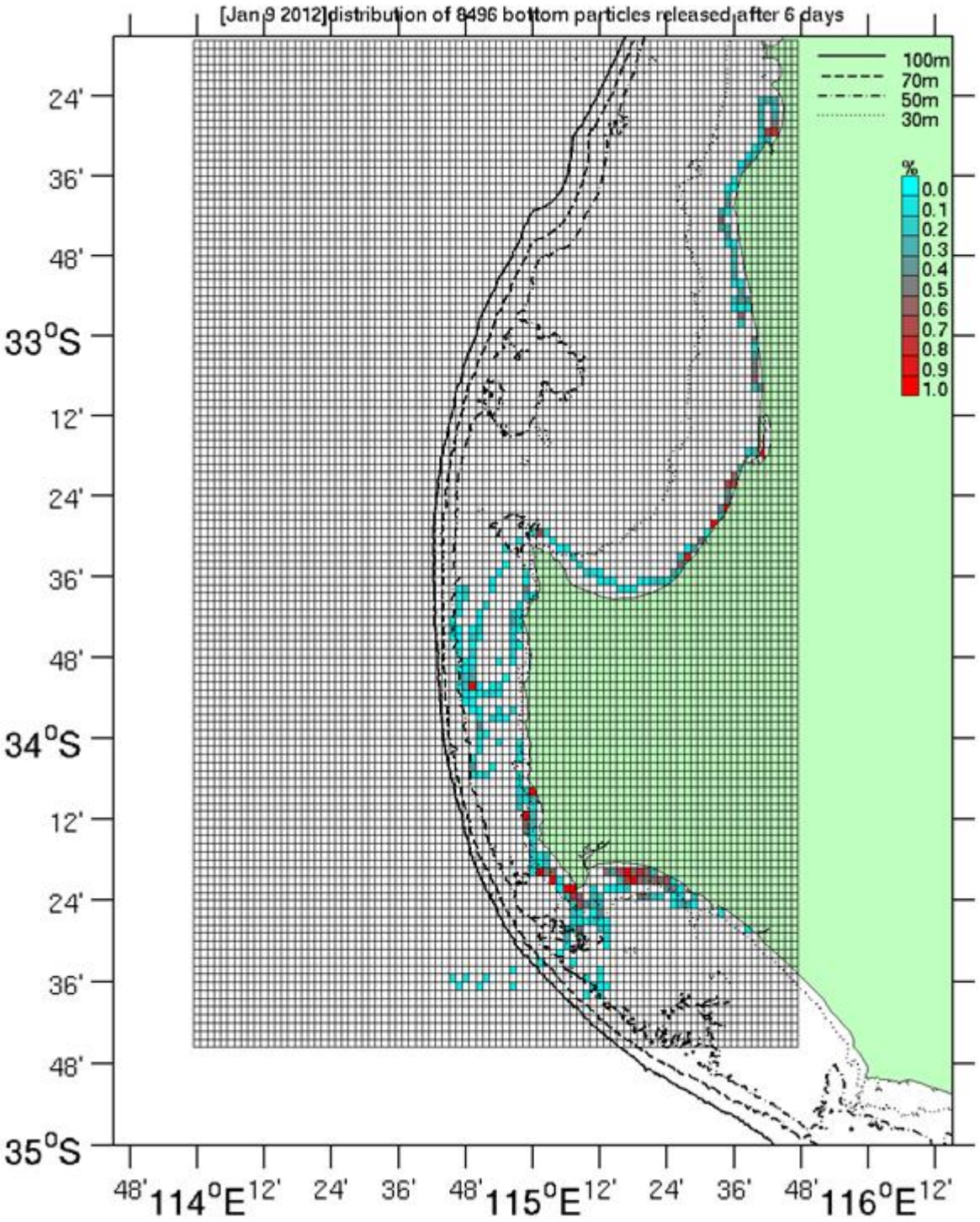
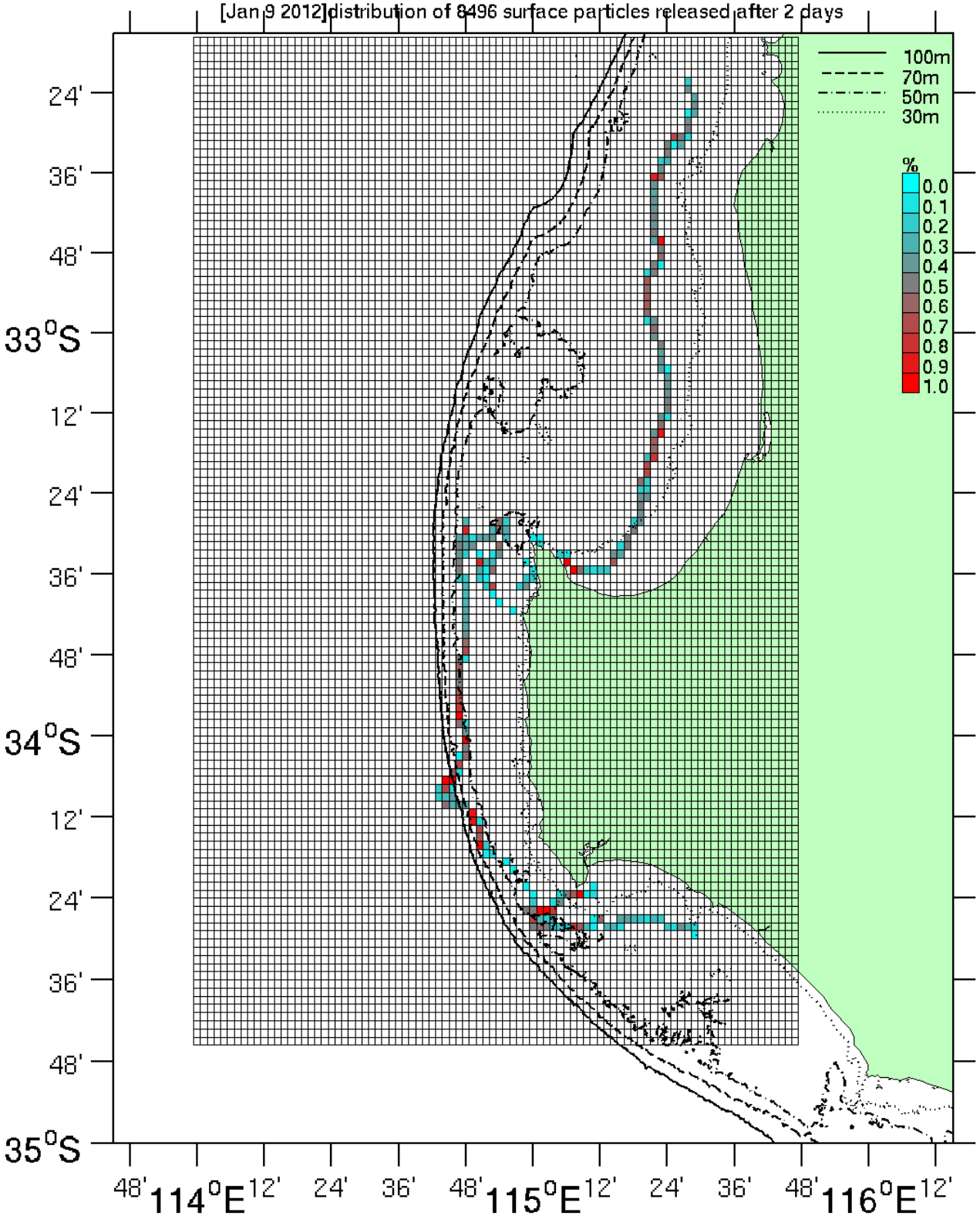


Figure 2 An example from model output for particles seeded 9 January 2012 on the bottom. The charts show the distribution and relative abundance of particles on 9, 13 and 15 January.100, 70, 50 and 30 m bathymetry is indicated. Grid indicates 2 x 2 km area.


[Jan 9 2012]distribution of 8496 surface particles released after 2 days
100m
70m
50m
30m
%
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
24'
36'
48'
33°S
12'
24'
36'
48'
34°S
12'
24'
36'
48'
35°S
48'
114°E
12'
24'
36'
48'
115°E
12'
24'
36'
48'
116°E
12'

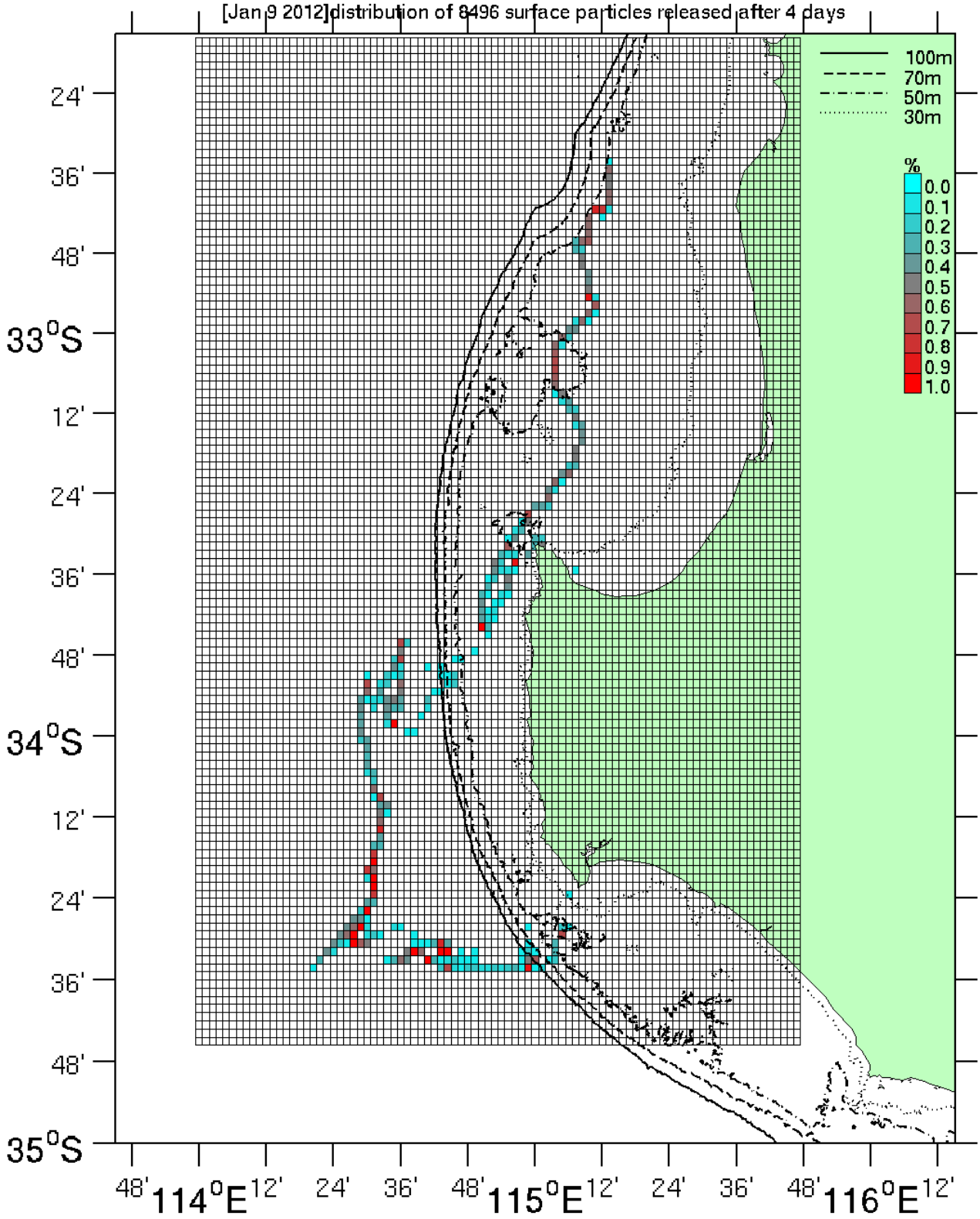


Figure 3 An example from model output for particles seeded 9 January 2012 on the surface. The charts show the distribution of particles on 11 and 13 January. 100, 70, 50 and 30 m bathymetry is indicated. Grid indicates 2 x 2 km area.

The model runs provided two useful insights. They showed general trends in movement of particles during the first few days of each cruise and provided locations of two by two km areas that yielded the highest chances of finding eggs and larvae (example 2 by 2 km grids marked in red on Figs 2 and 3). These “red grids” were the starting sampling points during each cruise and only red grids inside 60 m water depths were used. As the cruise progressed we also used data from the DNA test and information from drifters to decide on the location of our sampling stations.

DNA identification

Plankton samples were tested for *G. hebraicum* specific DNA. In the presence of *G. hebraicum* DNA in a sample, a PCR reaction was activated and resulted in the amplification of a 150bp DNA fragment that was visualised on an agarose gel. In the absence of *G. hebraicum* DNA no product was amplified. The test also included a second reaction that simultaneously amplified a larger DNA fragment in the presence of any fish DNA (ca. 250bp) (Figure 4).

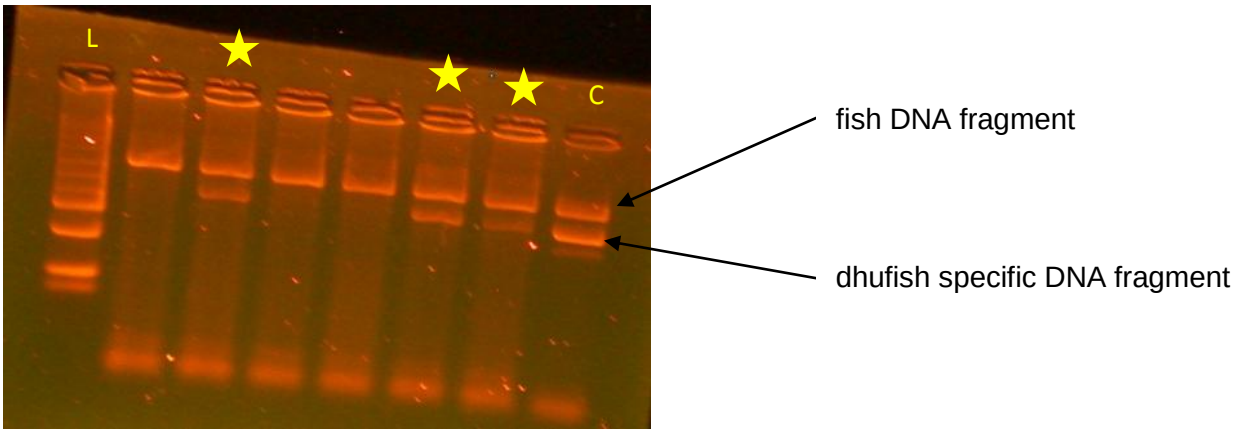


Figure 4 An example agarose gel. * indicates samples positive for *G. hebraicum*. C indicates the positive control, L indicates the size ladder. The remaining lanes were negative for *G. hebraicum*.

The universal DNA fragment was amplified from all plankton samples (n = 474). The *G. hebraicum* -specific DNA fragment was amplified from 92 plankton samples. All of the DNA sequences obtained from *G. hebraicum* -positive samples collected during January (n = 32 sequences) were identical and an exact match for *G. hebraicum* and had multiple mismatches with other candidate species, including *Glaucosoma* congeners (Figure 5). The phylogenetic analysis placed the plankton DNA sequences into a clade with *G. hebraicum* and to the exclusion of all other species with 100% bootstrap support (Figure 6).

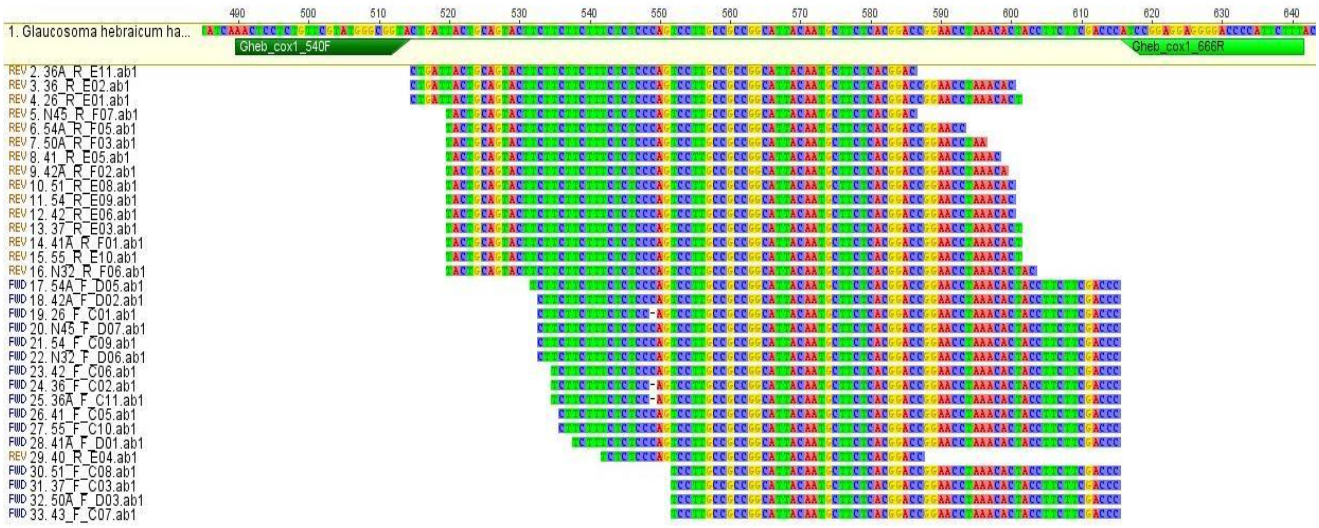


Figure 5 An alignment of DNA sequences obtained from mixed plankton samples collected during January 2012 (lower section (rows 2 to 33) against a *G. hebraicum* reference sequence (upper section, row 1). Note that all plankton sequences are identical and identical to the *G. hebraicum* reference sequence. Also illustrated are the positions of the PCR primers (Gheb cox1 540F and 666R) used to amplify the *G. hebraicum* -specific DNA fragment.

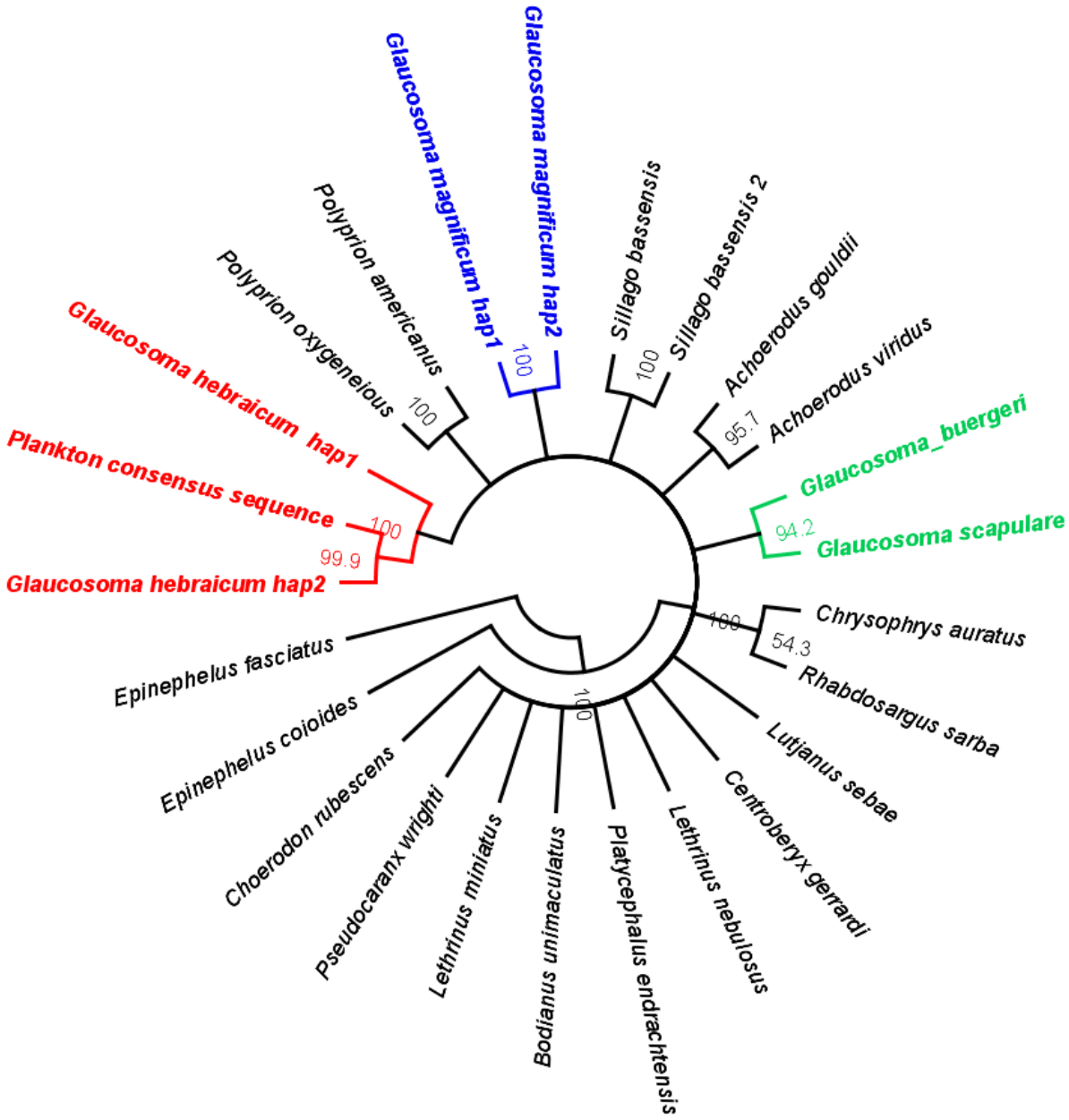


Figure 6 Neighbour-joining tree based on Tamura-Nei distances between 126 nt DNA fragments obtained from plankton samples and candidate fish species. Numbers at nodes indicate bootstrap support.

DNA quantification: quantitative PCR

Eighty seven of the 90 positive samples tested provided non-zero estimates of *G. hebraicum* DNA present in plankton samples. The three that failed may have been due to technical error in the reaction setup, or because the amount of DNA present was below the detectable threshold for this methodology, but they were not repeated. The mean concentration of *G. hebraicum* DNA obtained from plankton homogenates was 0.063 ng/µL (± 0.016 SE). Concentrations of *G. hebraicum* DNA obtained from spiked plankton samples ranged between 0.001 ng/µL and 0.122 ng/µL (Figure 7). The correlation between the first and second independent DNA extraction from the spiked samples was positive ($r = 0.734$) and highly significant ($p < 0.01$; Figure 8). The weight

of the tissue spiked into plankton samples was a significant predictor of the quantity of *G. hebraicum* DNA present in DNA extracts (adjusted $r^2 = 0.52$, $F = 17.104$, $p < 0.01$, df 1, 14; Figure 9).

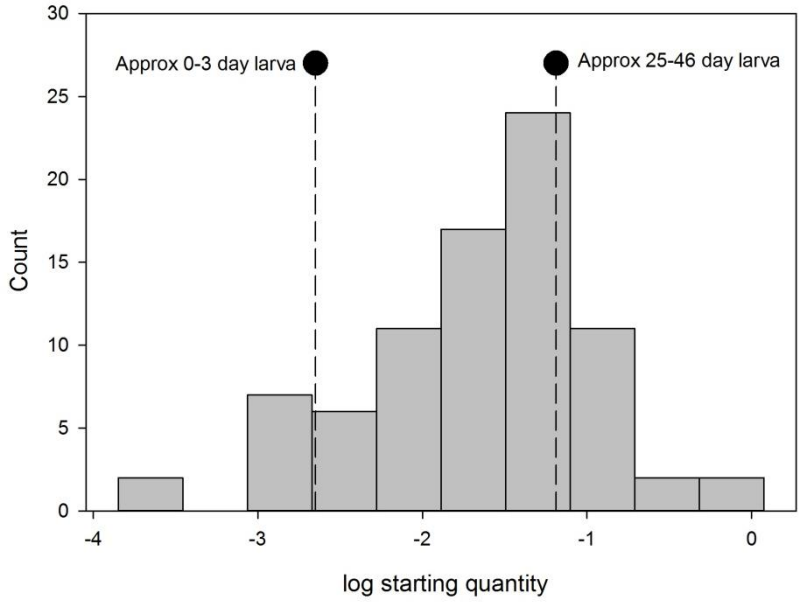


Figure 7 Distribution of concentrations of *G. hebraicum* DNA present in plankton samples (log ng/µL)

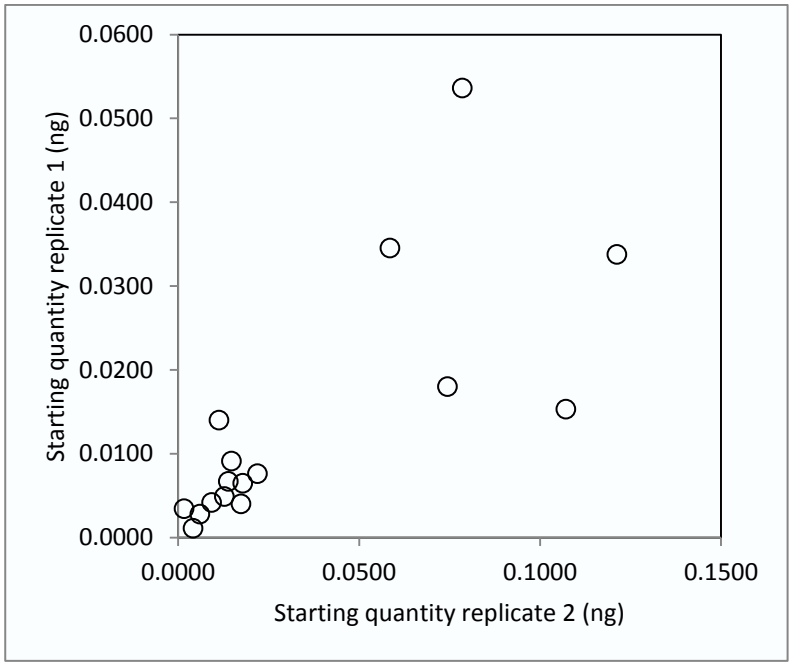


Figure 8 Correlation between the concentration in two independent DNA extractions from plankton samples spiked with *G. hebraicum* tissue.

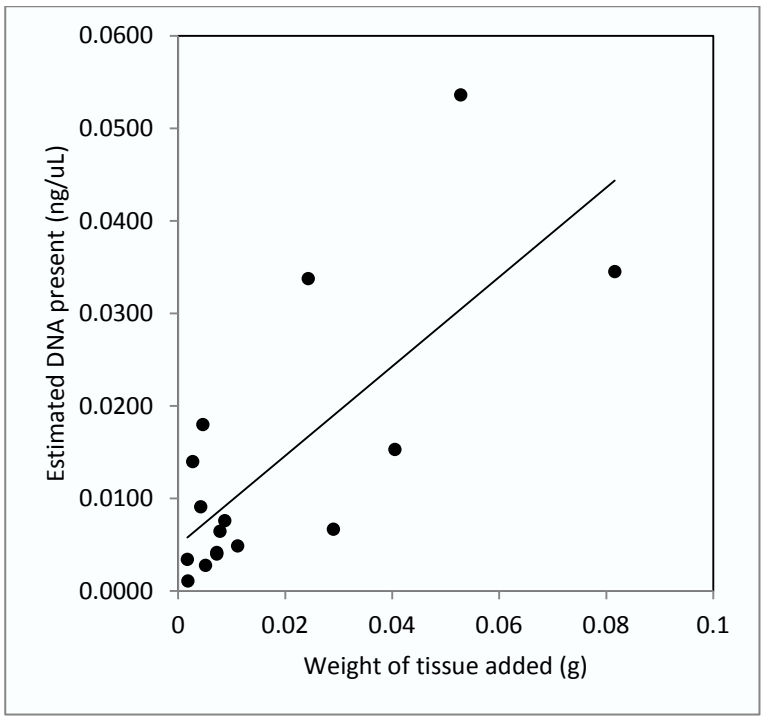


Figure 9 Relationship between the weight of *G. hebraicum* tissue added to plankton samples and the concentration present in DNA extractions.

Figure 10 and Figure 11 show the locations in the south-west and Metropolitan regions where plankton sampling was conducted and in which samples of *G. hebraicum* were identified via DNA methods.

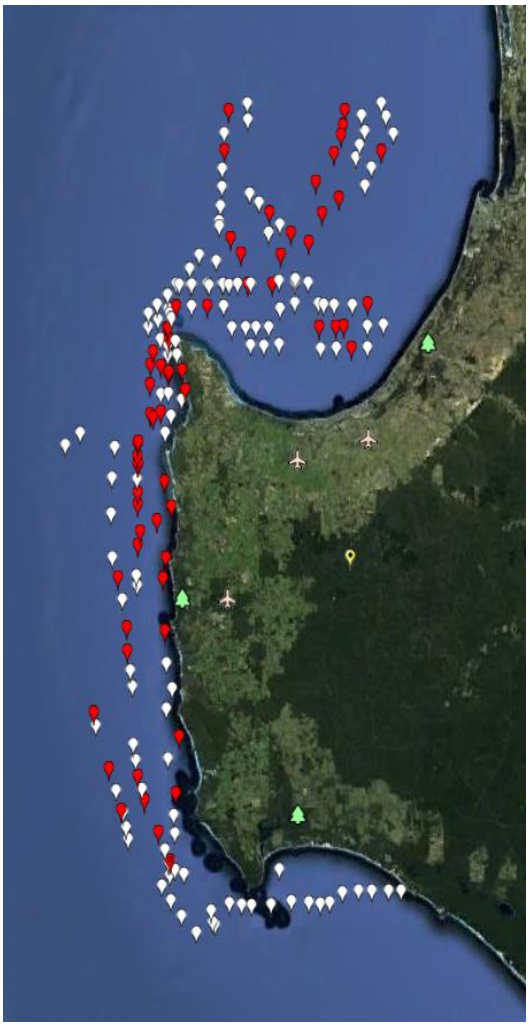


Figure 10 Map showing stations sampled in the Capes region (site 1). Red symbols indicate presence of *G. hebraicum* eggs or larvae (results based on DNA test) and white symbols indicate samples with no eggs or larvae.

Figure 11 Map showing stations sampled in the Perth metropolitan area NE of Rottnest Island (site 2). Red symbols indicate stations with eggs or larvae and white symbols indicate samples with no eggs or larvae.

Our sampling confirmed the presence of *G. hebraicum* DNA in plankton samples and thus eggs or larvae of this species in areas where its *G. hebraicum* juveniles occur.

We compared the biomass index from quantitative PCR among three months of sampling in the Capes region and stations sampled in metropolitan Perth waters in March and April. The means (± standard deviations) were 0.005 (±0.006), 0.043 (±0.032), 0.093 (±0.20) and 0.012 (±0.010) ng DNA uL sample for December, January and February and metropolitan Perth, respectively. This difference in biomass was significant (Kruskal-Wallis$_{df=2}$, p = 0.000) among Capes stations. The sampling effort was not uniform among the months with 48, 70 and 105 stations sampled in December, January and February, respectively, and these results need to be interpreted with caution. Sampling in metropolitan waters included only 20 stations and they were not included in the comparison.

Objectives:

2) Unravelling transport areas of eggs and larvae and correlating it with physical and biological processes

3) Predicting larval sources and sinks and relating it to currents, salinity, temperature, chlorophyll and food

Modelled circulation

Local hydrodynamic features typically have a great influence on the transport of eggs and larvae (Scheltema, 1986, Shanks, 1995). According to their intensity and direction, currents will either retain larvae in the vicinity of suitable settlement areas enabling recruitment or export larvae away from the spawning locations. If advected larvae are unable to reach suitable habitats to settle they will be lost from the population. Most larvae are too weak swimmers to move horizontally in the water column against strong currents however vertical migration allows larvae to maintain their position in the water column. There is no information on larval behaviour of *G. hebraicum* and consequently it has not been built into the present model.

Here we present an example of a model simulation carried out before the January 2012 cruise with particle release on 2 January. In this report, we focus on the model output during this period to discuss the shelf circulation structure.

In January 2012, there were four pulses of southerly winds (positive, northward wind stress), with peaks around the 8th, 16th, 23rd, and 29th of January (Figure 12). The northward velocity from

OceanMAPS responded to the wind forcing to generate northward surface current pulses of 15-20 cm/s. Northward surface currents of similar magnitudes were generated by the short simulation of the ROMS downscaling around 8th of January (Figure 12). However, the ROMS simulation provided a more detailed structure of the shelf circulation due to its high resolution, as shown below.

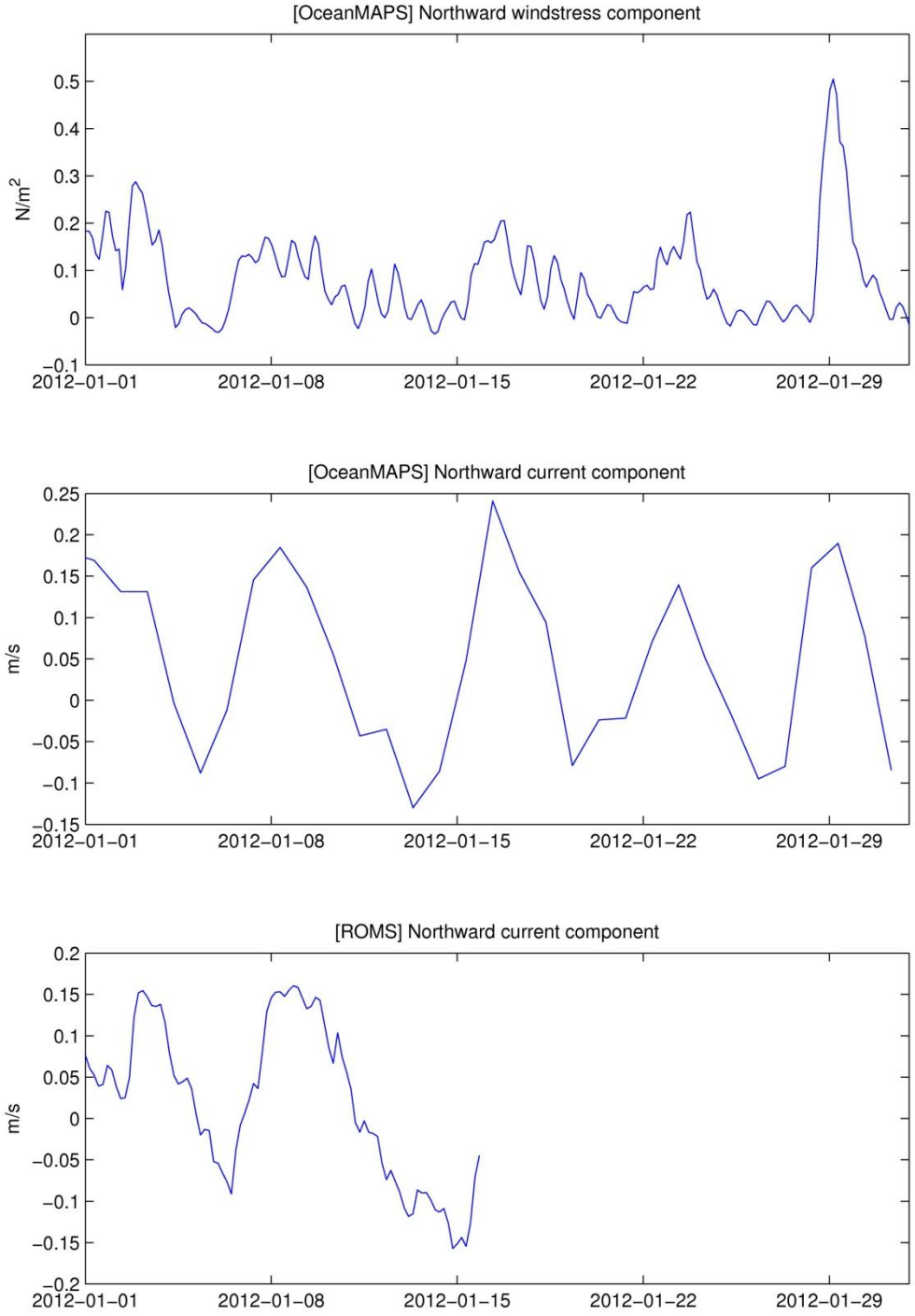


Figure 12 Northward wind stress, OceanMAPS surface current, and ROMS current averaged in the shelf area between Perth and Geographe Bay in January 2012.

Figure 13 shows a comparison between the daily-averaged surface wind stress on 8 and 10 January 2012. On 8 January, there were predominantly south-southeasterly winds along the coast

between Shark Bay and the Capes region, which is favourable to generate the Capes Current along the coast. After the peak southerly winds on 10 January, the surface winds started to weaken and veer to the offshore direction, so it is expected that the Capes Current would weaken.

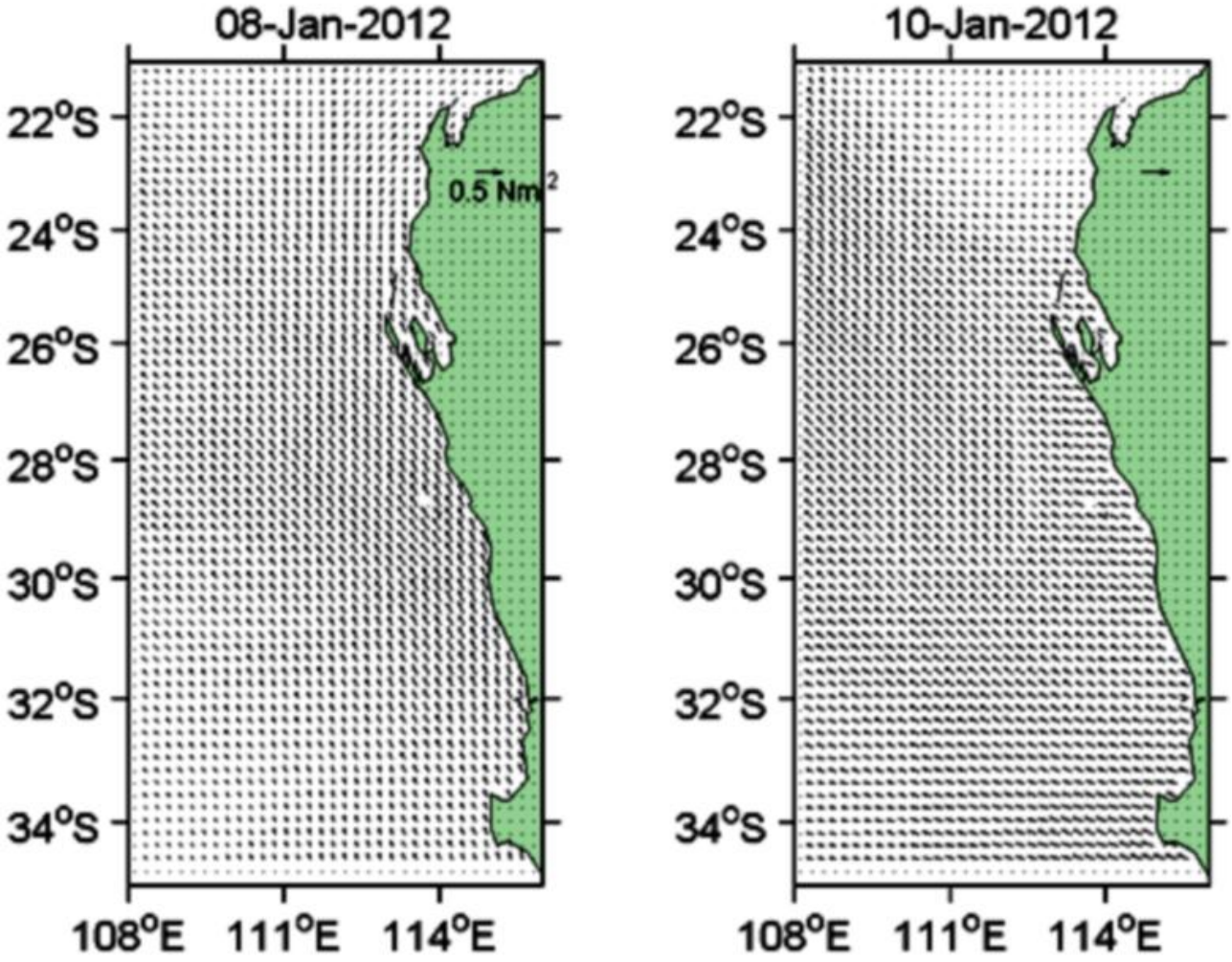


Figure 13 Daily averaged surface wind stress fields on 8 and 10 January 2012.

The ROMS model simulation captured a weak, eddying structure of the Leeuwin Current offshore and the Capes Current on the inner shelf on 8 Jan 2012 (Figure 14). Northward surface currents developed near Cape Leeuwin and flowed all the way up to the Shark Bay with current speeds up to 0.5 m/s. The northward currents off the Capes may not be well connected with the northward currents north of Geographe Bay, due to the discontinuity of the coastline. When the southerly wind relaxed on 10 January, the surface currents on the shelf weakened and veered offshore (Figure 14) and a stronger southward current (the Leeuwin Current) approached the shelf break.

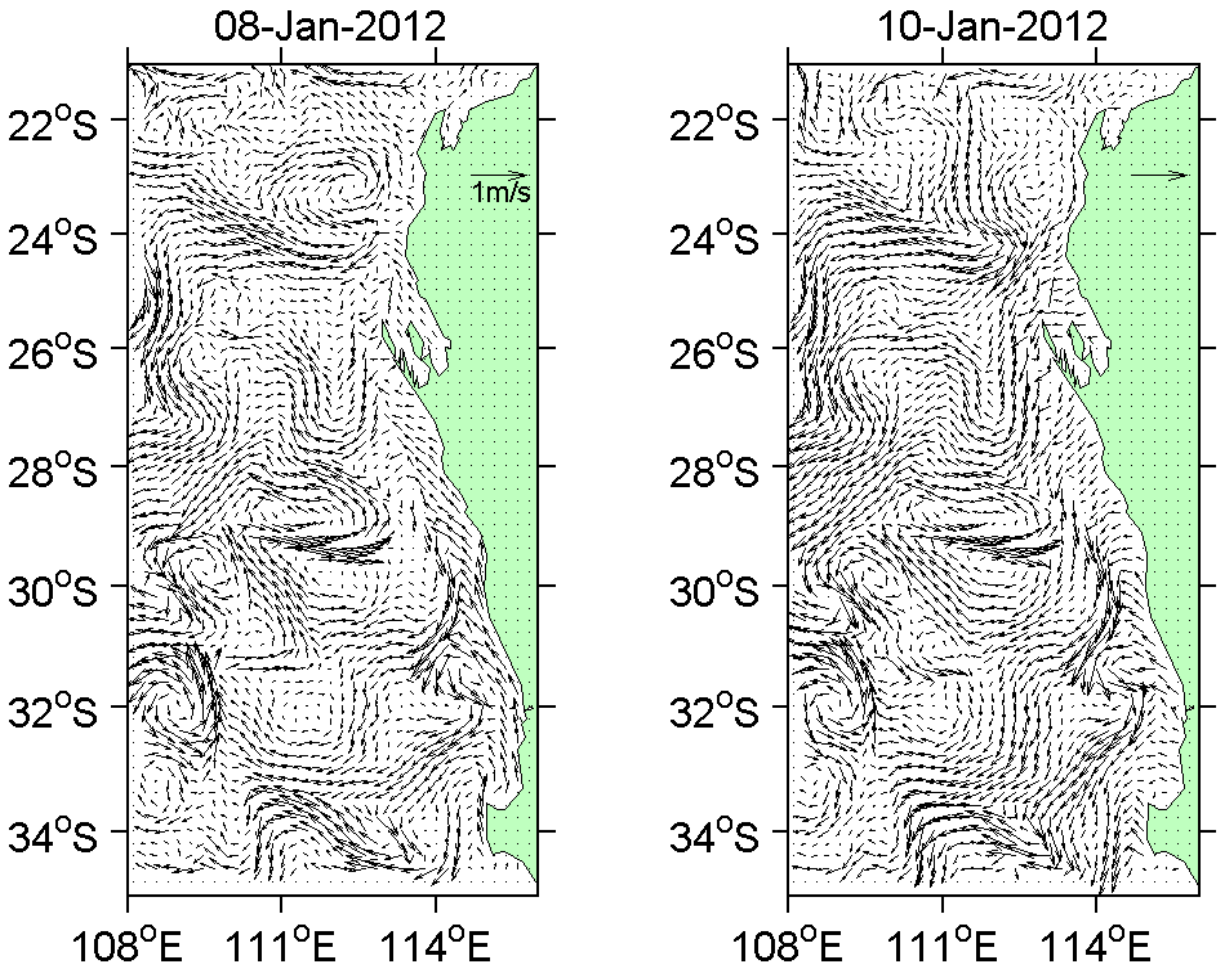


Figure 14 Daily averaged surface velocity on 8 Jan 2012 (left) and 10 Jan 2012 (right).

Figure 15 compares the vertical structure of the longshore and cross-shore velocities of the Capes Current along a transect near 32°S on 8 and 10 January 2012. The Leeuwin Current (negative velocity in blue) and the Leeuwin Undercurrent (positive velocity in yellow or red) were present offshore of the shelf break. Inshore of the Leeuwin Current was the Capes Current that flowed northward in the shallow water. The water in the coast region is shallow, usually 40m or less, and the whole water column moved northward. On 10 January, the Leeuwin Current was slightly stronger, while the Capes Current was slightly weaker, compared with 8 January.

The cross-shore velocity, however, showed a clear two-layer structure on the shelf, which was different from the vertical structure of longshore velocity. On 8 January, the surface water moved offshore due to the Ekman effect of the southerly wind forcing in the southern hemisphere, while in the bottom Ekman layer, water moved onshore to keep mass conservation. On 10 January, the surface offshore current extended further west, due to the offshore wind forcing; however, the onshore subsurface and bottom flows were still present to conserve the mass balance on the shelf. In the deep water, offshore flows were present in the Leeuwin Undercurrent depth.

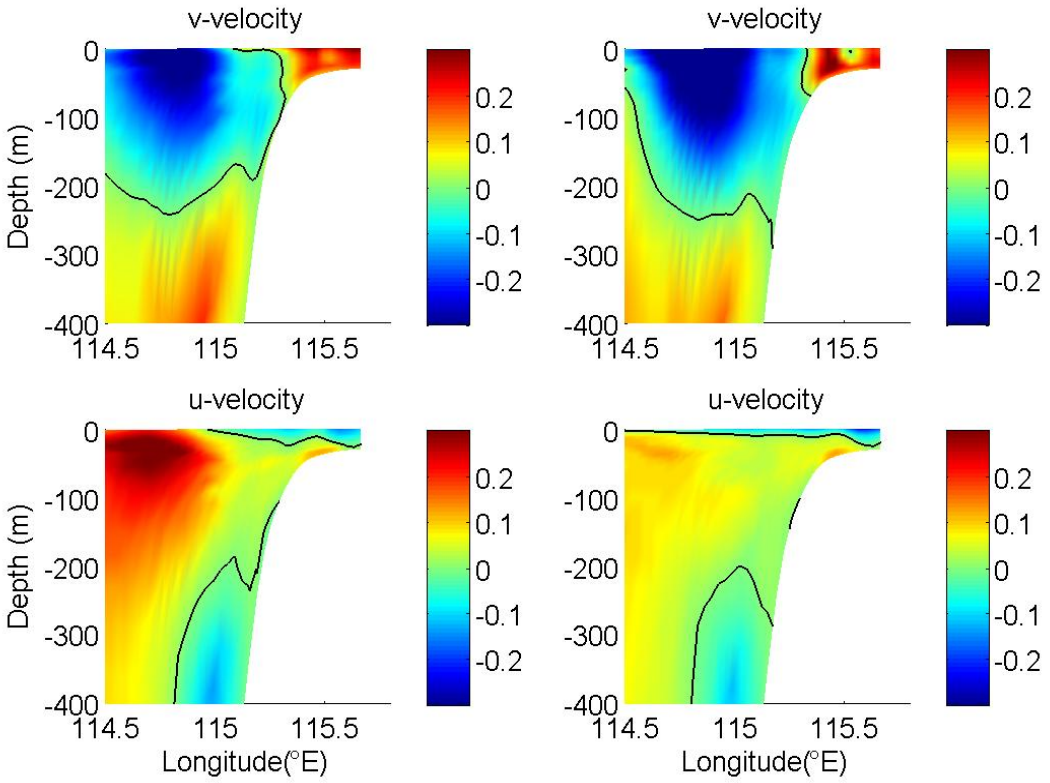


Figure 15 Longshore (upper panel) and cross-shore (lower panel) velocities along a transect near 32ºS on 8 Jan 2012 (left panel) and 10 Jan 2012 (right panel). Positive longshore velocity is northward and positive cross-shore velocity is eastward towards the coast. The black contour lines are zero velocities

Particle tracking

The cross-shore current in the coast region is much weaker than the longshore current; however, the model results suggest that it is very important for the shelf retention and particle transport. Particles released at the surface layer tended to follow the offshore surface current and move offshore; while particles released in the bottom layer tended to follow the onshore bottom flow to converge to shallow waters (Figure16).

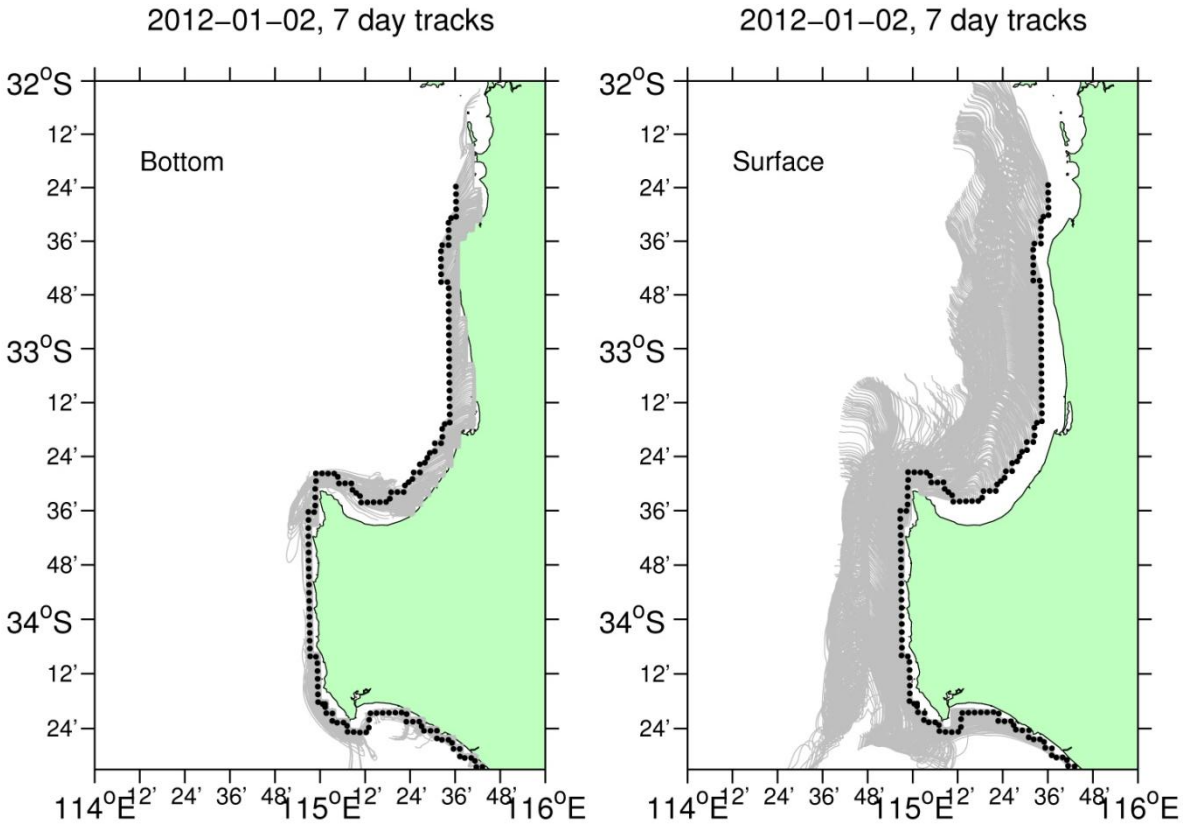


Figure16 7-day trajectories of particles released at the bottom and surface layers of the model on 2 January 2012. Black dotted line shows sites of particle release.

There was regional heterogeneity in terms of the onshore/offshore movement of the passive particles. For example particles released on 2 and 9 January 2012 in the surface layer showed rapid offshore advection except for particles around both capes (Figure 17). Based on this we suggest that these locations represent possibly retention areas for eggs and larvae.

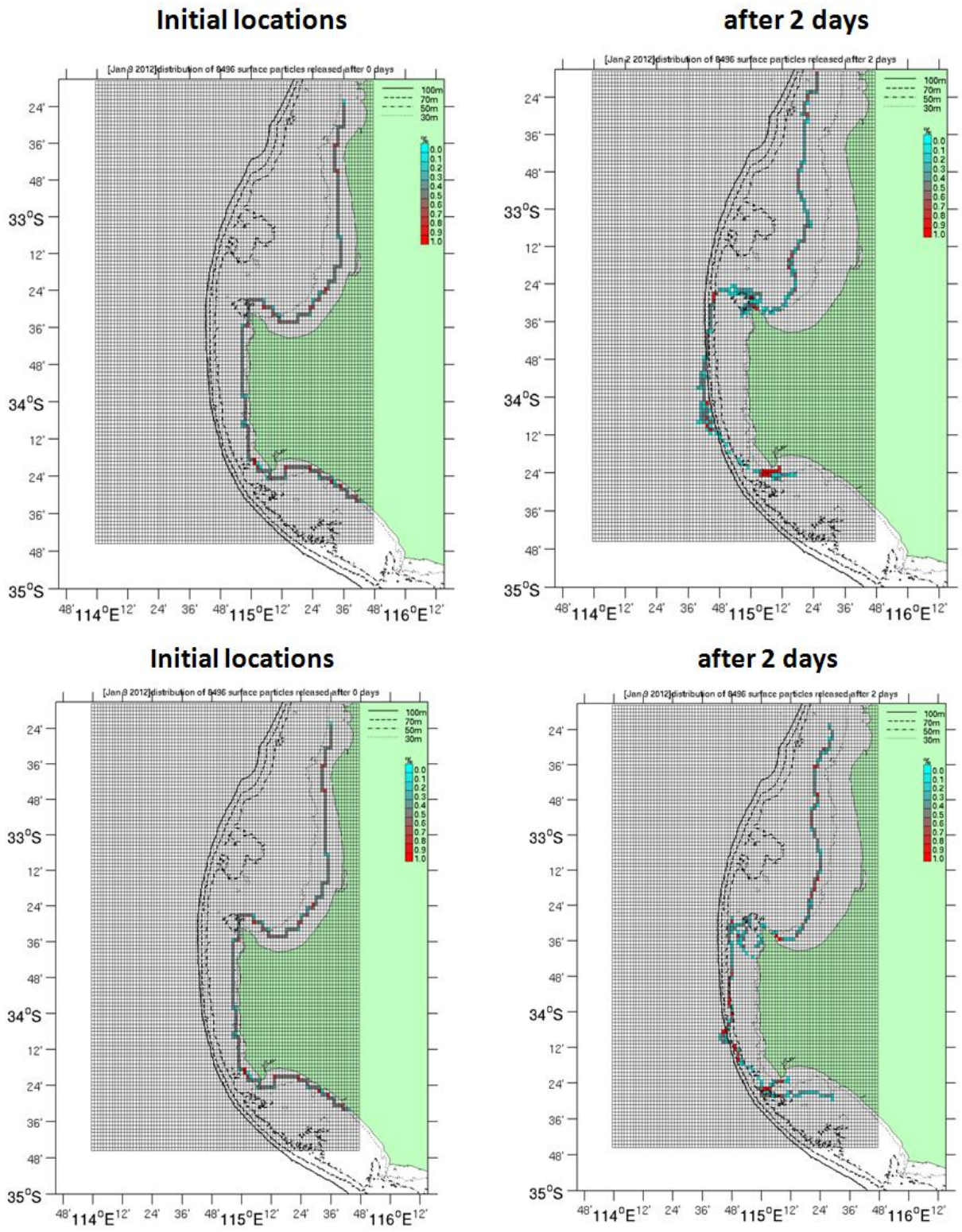


Figure 17 Particles released at surface on 2 and 9 January 2012 (upper and lower panel, respectively).

In summary, the particle (larvae) tracking model can provide information relevant to the early life history of *G. hebraicum* if properly interpreted. Results show that eggs/larvae in nearshore habitats can be rapidly carried offshore by surface Ekman flows. Larvae tend to congregate around Cape Naturaliste and Cape Leeuwin after 2 days. Onshore return flows near the sea floor may help larval retention when the larvae vertically migrate. Larval behaviour of *G. hebraicum* is not well known. Based on eye development *G. hebraicum* larvae may initially be restricted to brightly lit

surface waters. After a week cones become longer and after 3 weeks rods increase allowing fish to feed in deeper waters suggesting adaptation to settlement at the bottom (Shand et al, 2001). The northward currents off the Capes will transport larvae north but may not be well connected with the northward currents north of Geographe Bay, due to this discontinuity of the coastline and the larvae in Geographe Bay may be retained there.

Observed circulation

Satellite images of sea surface temperature showed cooler water flowing along the coast from December 2011 to February 2012 indicative of the Capes Current (

Figure 18). Parts of Geographe Bay were not under the influence of this current and larvae located there are unlikely to have been transported north. This shows well on satellite images of sea surface temperature (

Figure 18). There was no significant difference among sampling months in the magnitude of the northward wind stress component that generated the Capes Current (ANOVA $_{df=2, F=0.706}$, p = 0.494) with means 0.09 (±0.07s.d.), 0.08 (±0.07 s.d.) and 0.09 (±0.07 s.d.) N m^{-2} for December, January and February respectively. January had 42 three hour periods versus 20 in other months when northward wind stress was zero or negative (Figure 19). However, the longest consecutive periods of no stress were 24 hours in January and 27 hours in January and February which would not have been long enough to downspin the Capes Current. Consequently, all larvae with the exception of the ones in Geographe Bay would have been transported north by the current.

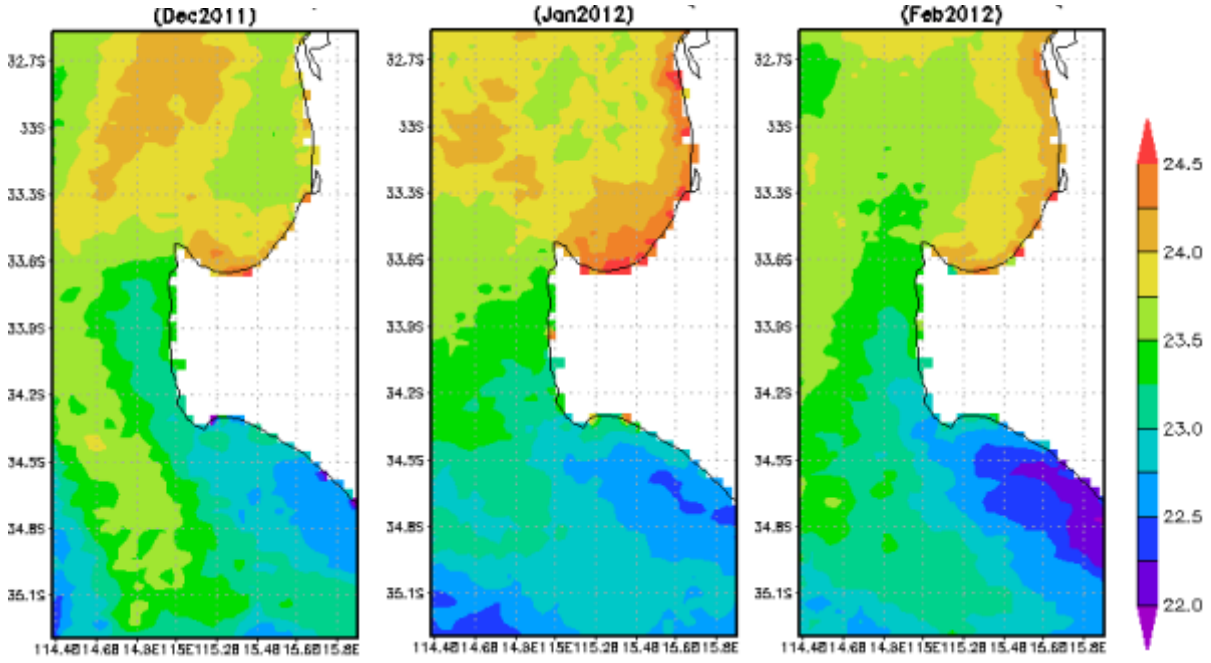


Figure 18 Satellite images of sea surface temperature along the south-western Australian coast showing cooler waters indicative of the Capes Current (note different temperature scale).

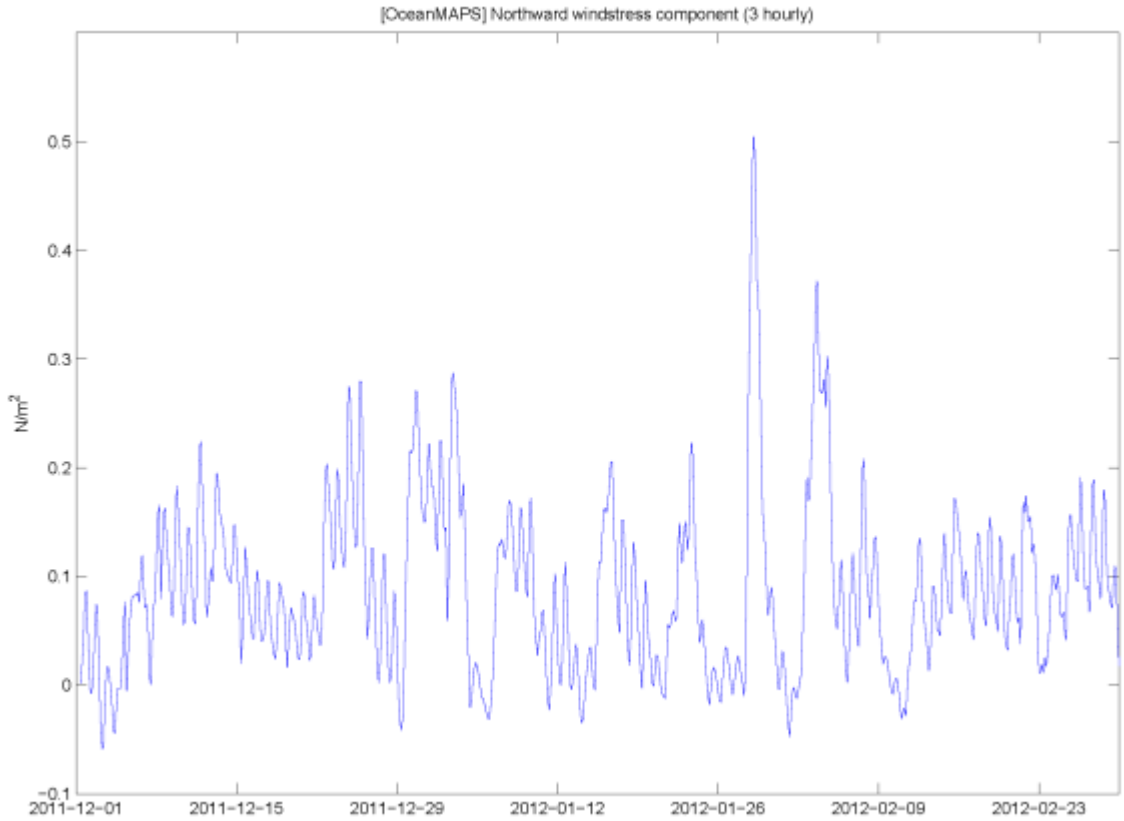


Figure 19 Northward wind stress from December 11 to February 2012

For convenience, the drifter tracks have been plotted in pairs as they were released.

Drifters #6670 and #8060, released 18th and 19th January 2012

These 2 drifters were deployed just southwest and east of Cape Naturaliste (Figure 20). #8060 drifted westwards to round Cape Naturaliste and then both drifters moved southwards (against the traditional north-flowing Capes Current). Drifter #8060 ran aground near Cowaramup, but #6670 continued southwards beyond Cape Leeuwin to leave the continental shelf and subsequently became entrained into an eddy field associated with the Leeuwin Current. Current speeds were quite variable, up to about 50 cm s^{-1} on the shelf but increased to 80 cm s^{-1} in the Leeuwin Current. Any larvae in the current near Cape Naturaliste would have rounded Cape Leeuwin within 5 or so days.

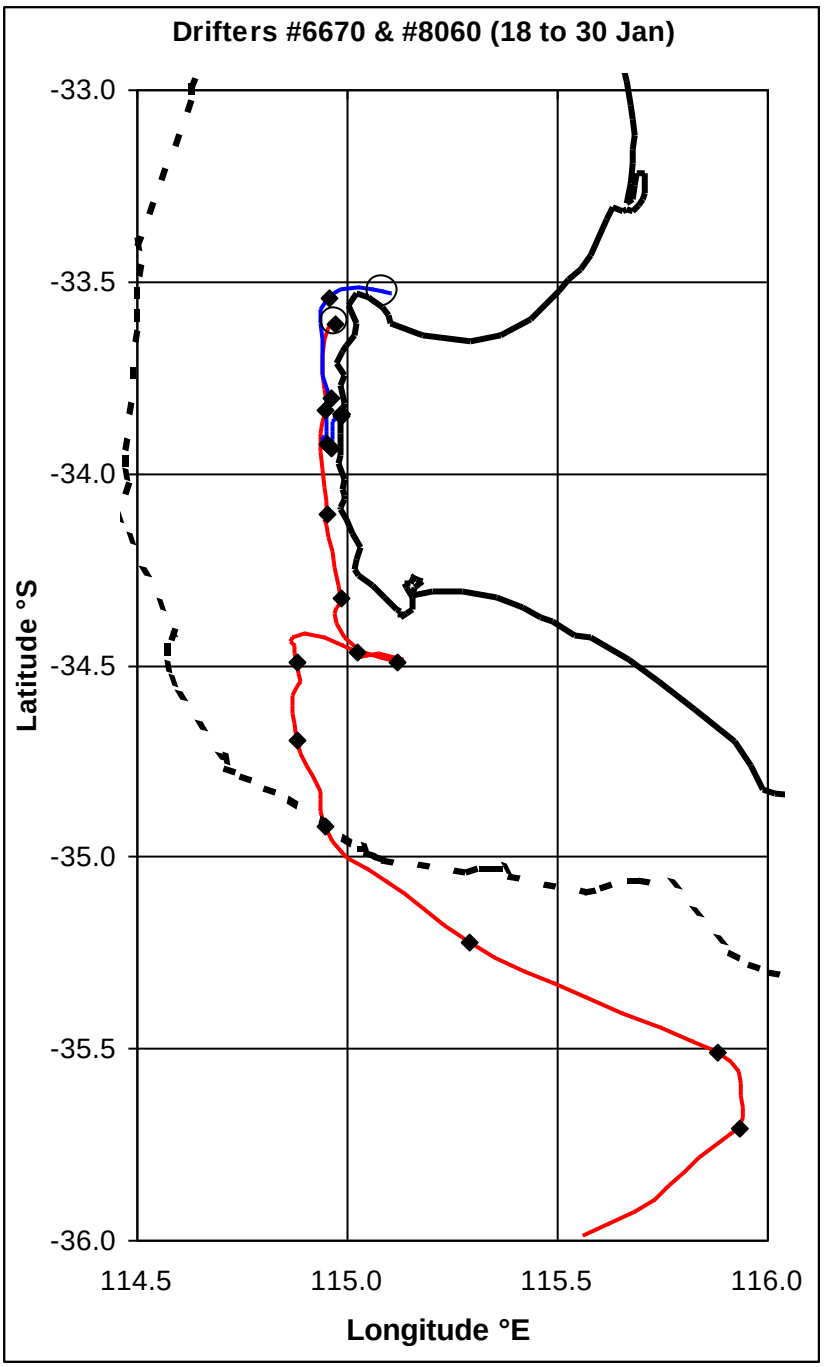


Figure 20 Drift trajectories of drifters #6670 (red) and #8060 (blue) released on 18th and 19th January respectively. The solid black line is the coastline, and the dashed black line the 200 m isobath (the nominal edge of the continental shelf). The circles denote the release positions of the buoys.

Drifters #9660 and #0660; released 15th February 2012

Drifter #9660 was deployed in southern Geographe Bay and #0660 just off Cape Naturaliste (Figure 21). Both initially moved inshore towards Bunbury before heading northwards up the coast in the Capes Current.

The initial current speed of #0660 was 30 to 40 cm s^{-1} but after a day dropped substantially and neither buoy exceeded 15 cm s^{-1} thereafter.

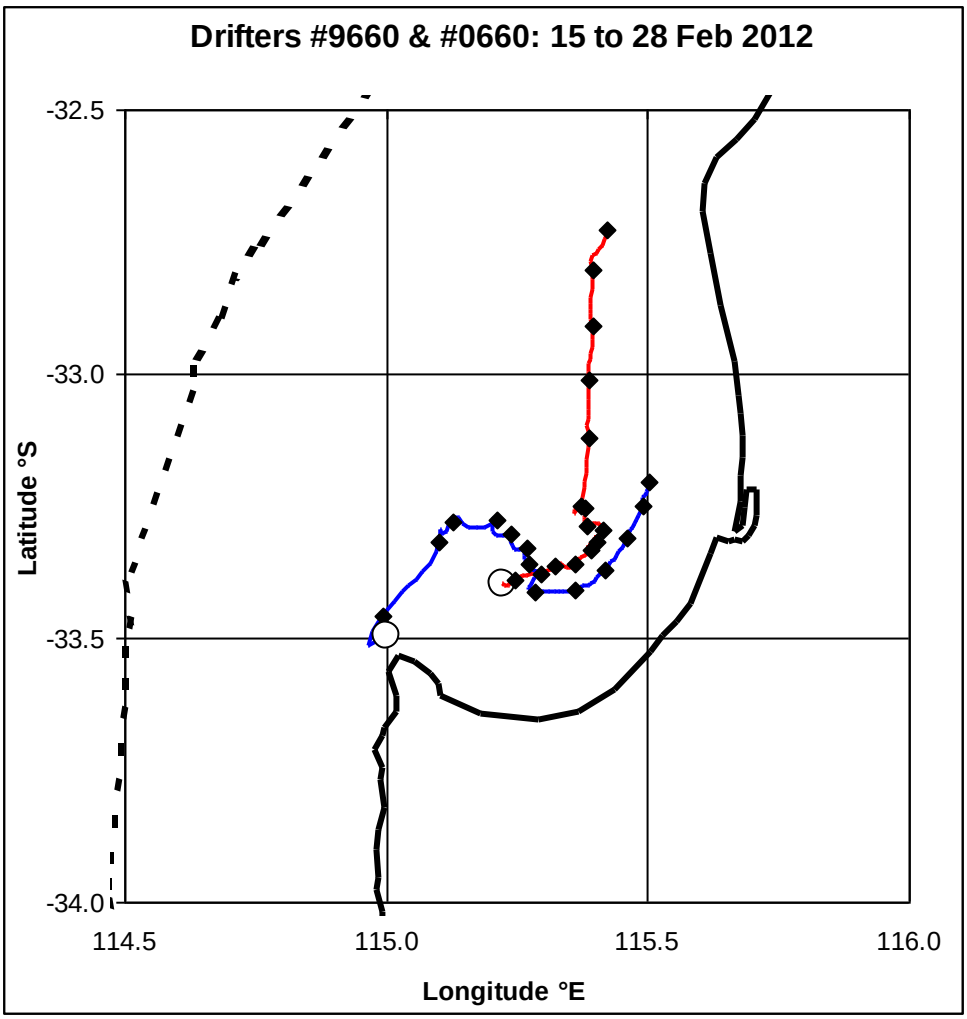


Figure 21 Drift trajectories of drifters #9660 (red) and #0660 (blue) released on 15th February. The solid black line is the coastline, and the dashed black line the 200 m isobath (the nominal edge of the continental shelf). The circles denote the release positions of the buoys.

Drifters #2670 and #1690; released 16th February 2012

These drifters were released along the inner- to mid-shelf southwest of Cape Naturaliste (Figure 22) and both initially headed northwards in the Capes Current. However, their paths soon diverged, with the inner drifter #1690 continuing northwards while after some 4 days the outer buoy #2670 moved slightly offshore to become entrained into the south-flowing current for another 4 days and then moved back inshore and resumed northward flow – it effectively returned to the release point of 2 weeks previously. Any larvae following this trajectory would have been retained in the Capes region for this period. The maximum current speed was 50 cm s^{-1} (1 knot) southwards.

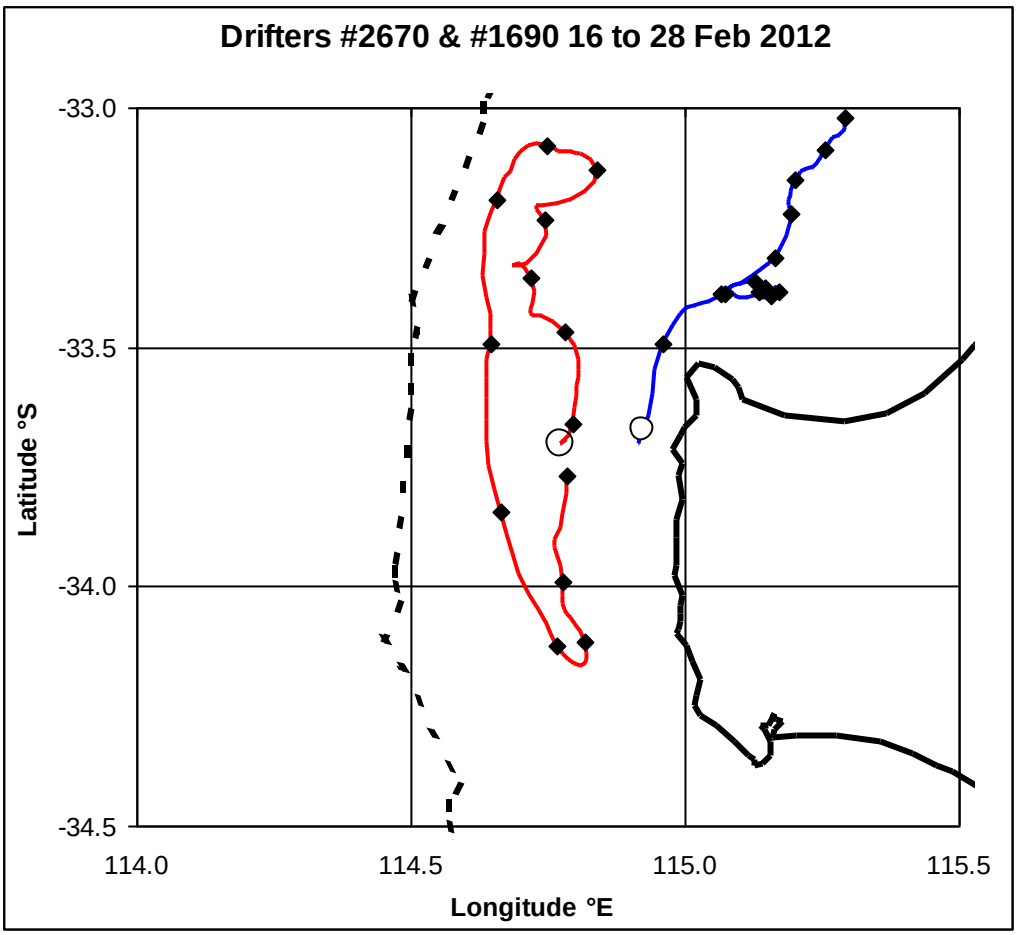


Figure 22 Drift trajectories of drifters #2670 (red) and #1690 (blue) released on 16th February. The solid black line is the coastline, and the dashed black line the 200 m isobath (the nominal edge of the continental shelf). The circles denote the release positions of the buoys.

Drifters #1650 and #5440; released 17th February 2012

Both drifters were deployed west of Cape Freycinet. The outer drifter #5440 (Figure 23) moved southwards for 3 days, then headed back up to the release point where it circulated in a small (10 km diameter) eddy. Thereafter it drifted northwards towards Cape Naturaliste in the Capes Current -- alongshore current speeds in this last phase were between 8 and 40 cm/s. Drifter #1650 remained near its release point for a week, then also moved northwards (parallel to #5440) in the Capes Current at 25 to 35 cm s^{-1}; there were some unexplained gaps in the transmission during this time.

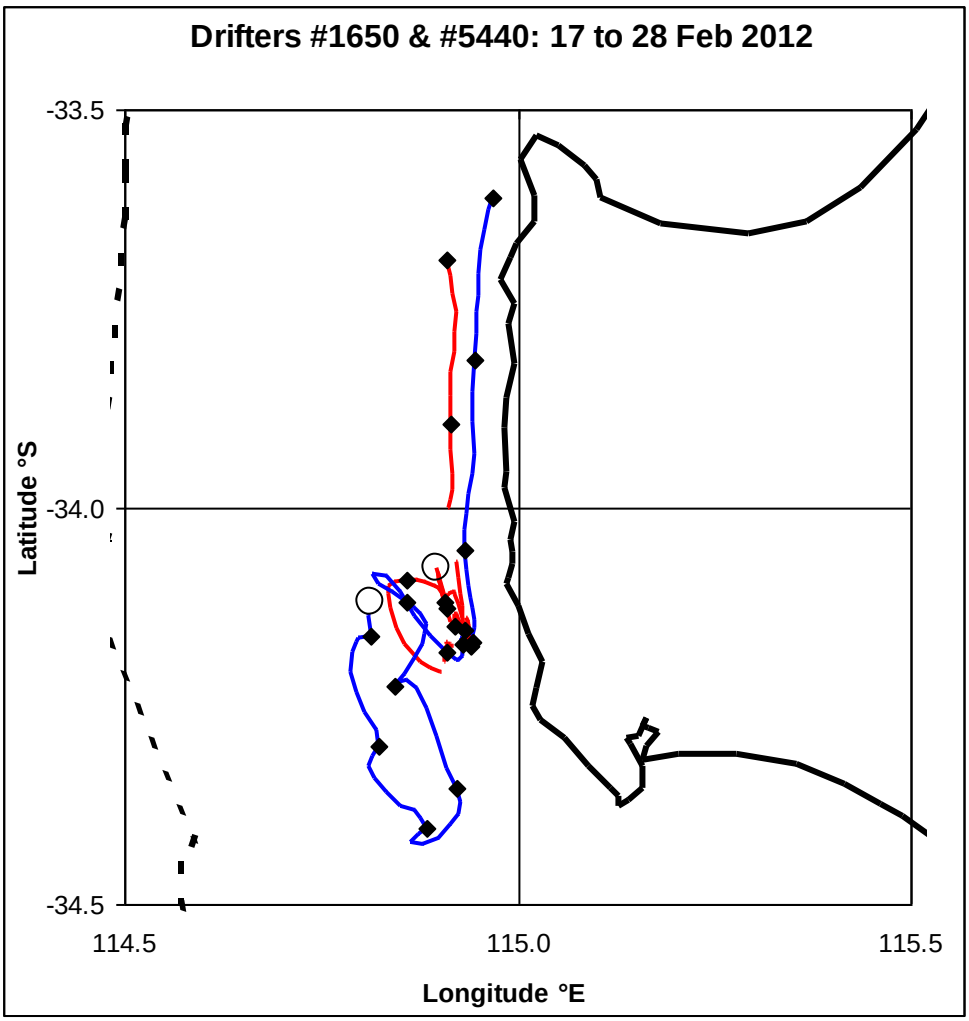


Figure 23 Drift trajectories of drifters #1650 (red) and #5440 (blue) released on 17th February. The solid black line is the coastline, and the dashed black line the 200 m isobath (the nominal edge of the continental shelf). The circles denote the release positions of the buoys.

Drifters #7660 and #3440; released 20th February 2012

These drifters were released on the inner continental shelf southwest of Cape Leeuwin. Drifter #7660 effectively remained trapped in a recirculation pattern at a generally constant latitude and an east-west excursion of about 30 km until 5th March (after the end of the survey cruise); current speeds were generally small but on one occasion reached 50 cm s^{-1} (Figure 24**)**. The second drifter #3440 also remained in this vicinity for a few days, then moved northwards past Cape Freycinet in the Capes Current at speeds of mainly 20 to 40 cm s^{-1}.

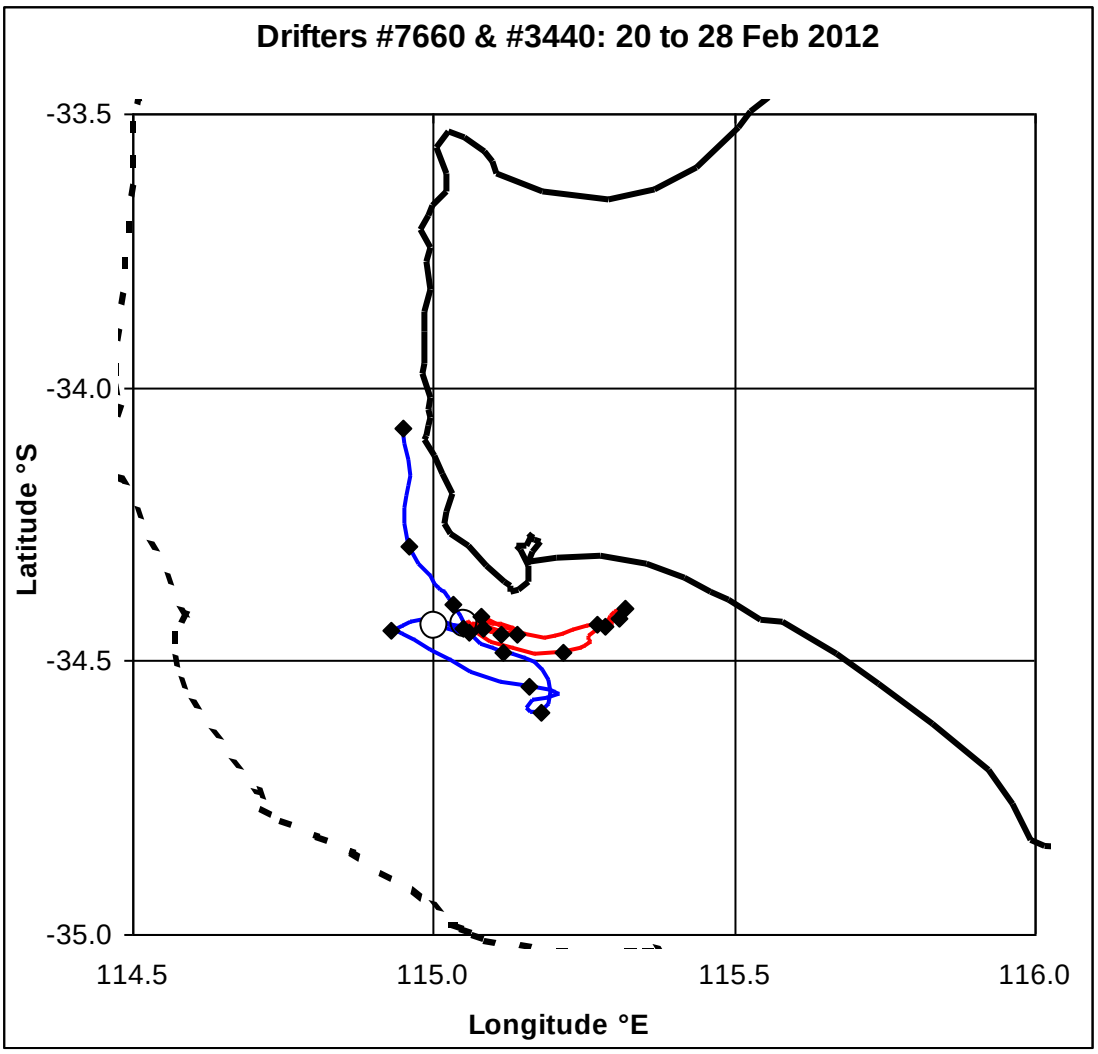


Figure 24 Drift trajectories of drifters #7660 (red) and #3440 (blue) released on 20th February. The solid black line is the coastline, and the dashed black line the 200 m isobath (the nominal edge of the continental shelf). The circles denote the release positions of the buoys.

The two ocean currents which largely governed the drifter movements were the Capes Current, which is the wind-driven northward flow operating along the inner continental shelf during the summer months (Pearce & Pattiaratchi 1999, Gersbach et al 1999), and the Leeuwin Current -- the stronger southward boundary current which tends to flow along the outer continental shelf and upper slope region. In the Capes area, in particular, variations in the relative positions (inshore-offshore) of these two dominant current systems will play a crucial role in the dispersal of larvae, and the cross-shelf exchange of water masses shown by some of the drifters can signify the difference between larval retention (and presumably survival to recruitment) and offshore dispersion (ultimate loss to the system). Those larvae which become entrained into the Leeuwin Current are likely to be carried rapidly southwards/eastwards towards the Great Australian Bight, although as shown in previous drifter studies (*e.g.* Pearce et al 2009) they may also be carried offshore by eddy currents and then swept north-westwards in the West Australian Current.

The trajectories of the drifters have amply demonstrated the high level of current variability (both spatially and temporally) on the continental shelf (see also Caputi et al 2010), with consequent implications for egg and larval transport. While the north-flowing Capes Current was evident for much of the time (Figure 21, Figure 22 and Figure 23), there were occasions when the drifters either moved southward along the coast (*e.g.* Figure 20) or became trapped in an apparently "dead-water" region just south of Cape Leeuwin (Figure 24). In this area a large number of *G. hebraicum* juveniles were observed recruiting to artificial habitats (Fairclough, unpublished data). It is worth noting that reliable anecdotal observations from a fishing boat in 20 to 30 m water depth off Cowaramup in January and February of the past 3 years have indicated that a 1-2 knot southward current can be found under strong easterly wind conditions, while on other occasions there was no observable drift (Jim Penn, pers. comm.), confirming the absence of the Capes Current shown by some of the drifters.

The current speeds were also very variable, ranging from virtually zero at times to the highest recorded speed of 83 cm/s in the Leeuwin Current south of Cape Leeuwin (Figure 20). Probably more relevant for present purposes were the current speeds on the continental shelf, which often exceeded 50 cm s^{-1} (~1 knot). Using the approximate "rule-of-thumb" that 1 cm s^{-1} ~~ 1 km/day, these peak water movements would have transported eggs and larvae some 50 km in a day.

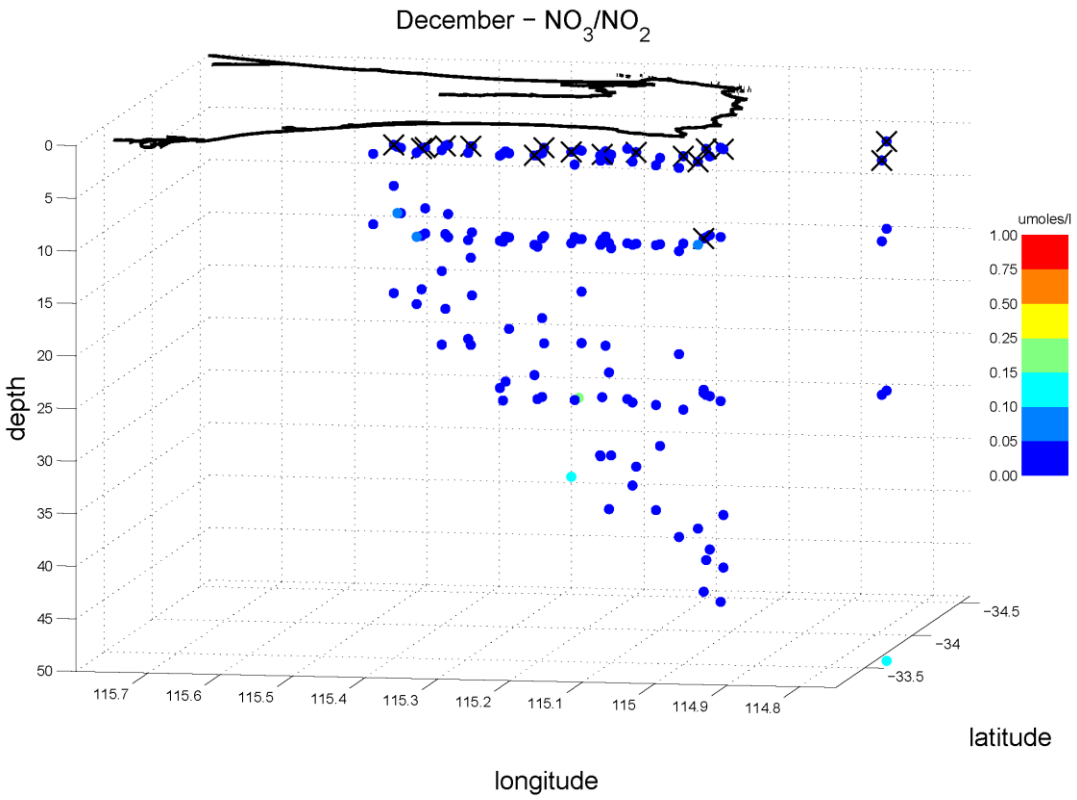
Correlation with environmental variables

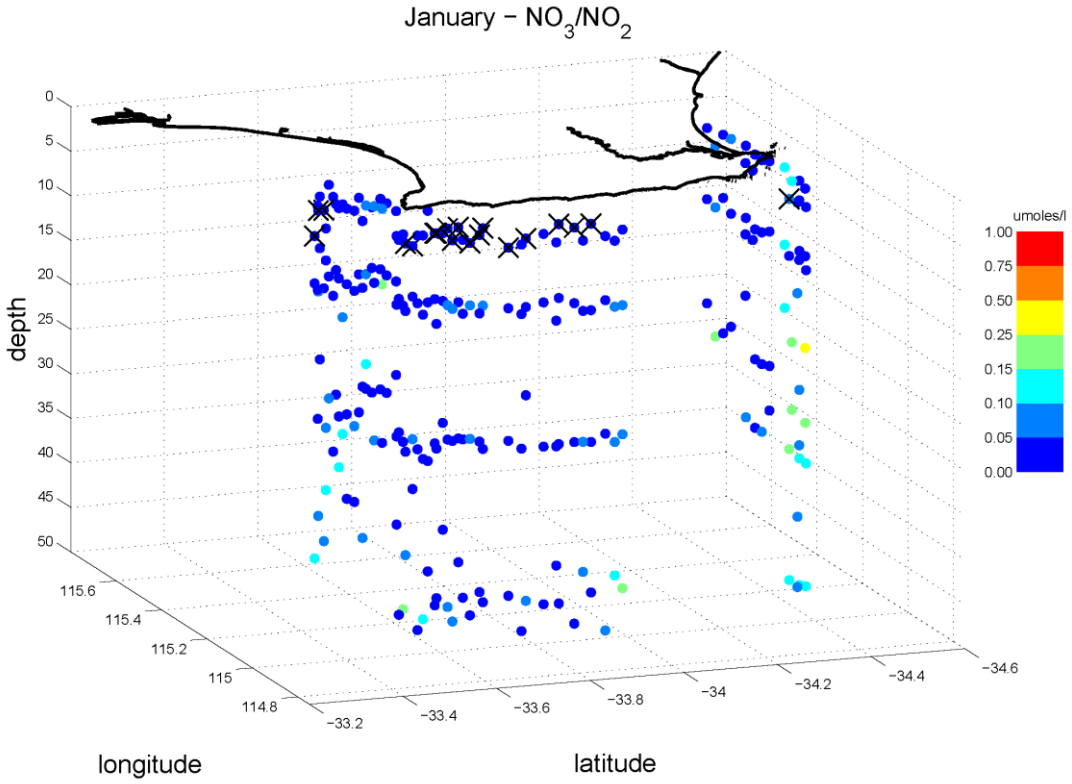
We examined the relationship between biomass index from quantitative PCR and temperature, salinity, nutrients (nitrite/nitrate, phosphate, silica, and ammonia) and chlorophyll. Nutrients in the Capes region were, in general, low, reflecting the oligotrophic environment in the south west of Western Australia. However, there were striking differences from month to month in nitrite/nitrate, chlorophyll and contribution of large phytoplankton to biomass (Table 7). There was evidence of substantial mixing of nutrients through the water column with some lower concentrations of nitrate in subsurface layers.

December was characterised by cooler and less saline waters (Table 7) 1.5 and 2 ºC cooler than January and February, respectively. Salinity increased only slightly from 35.45 in December to 35.54 and 35.53 PSU in January and February respectively.

In December and January nitrite/nitrate mean concentrations were 0.01 (± 0.01 s.d.) and 0.03 µmol L^{-1} (± 0.04 s.d.), respectively. In February the concentration was an order of magnitude higher with a mean of 0.11 µmol L^{-1} (± 0.14 s.d.). The difference was highly significant ($p = 0.000$, $Anova_{(df\ 2,\ F=21.420)}$). In December nitrite/nitrate was mostly below the detection level in all stations at all depths (Figure 25). In January and February there was higher concentration in the vicinity of Cape Leeuwin and nitrate was transported northwards in the Capes Current (Figure 25). Higher wind strength (Figure 19) at the end of January/beginning of February is likely to have caused seasonal

upwelling in the Capes region. Upwelled waters contained relatively high nitrate concentrations (>0.5 µmol L^{-1}) (Figure 25). Evidence of summertime upwelling in the Capes region has been identified previously (Gersbach et al, 1999) and associated with higher primary production rates (Hanson et al, 2005).





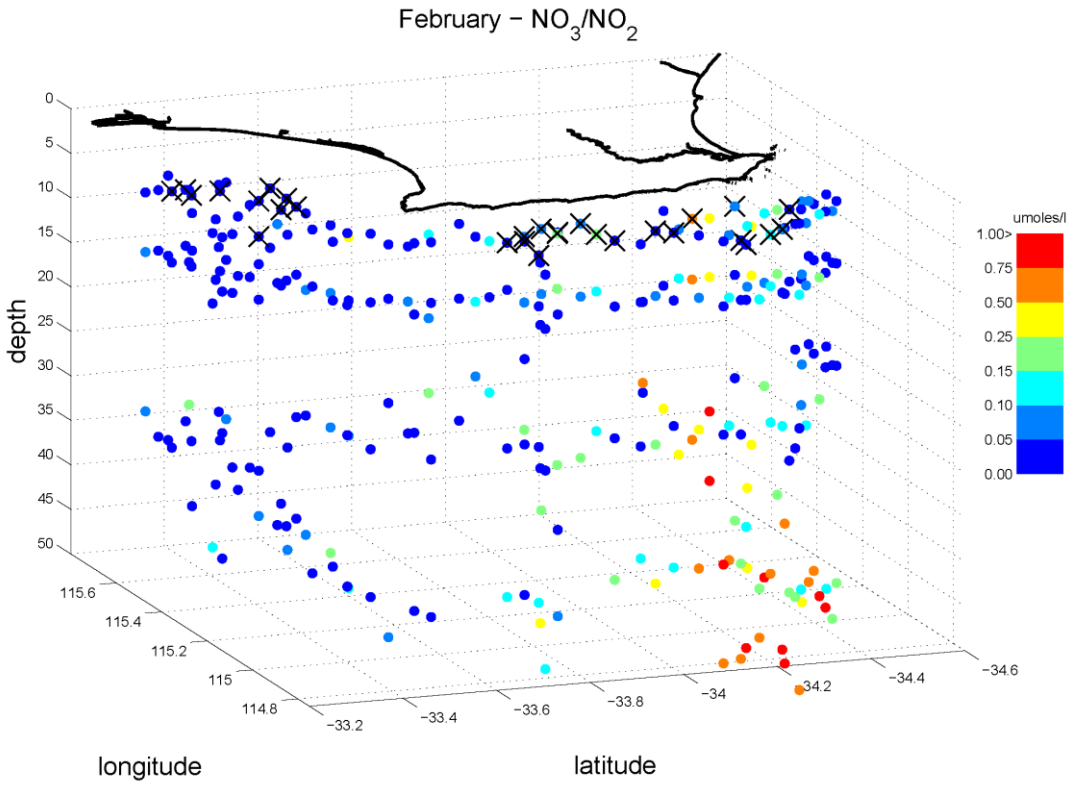
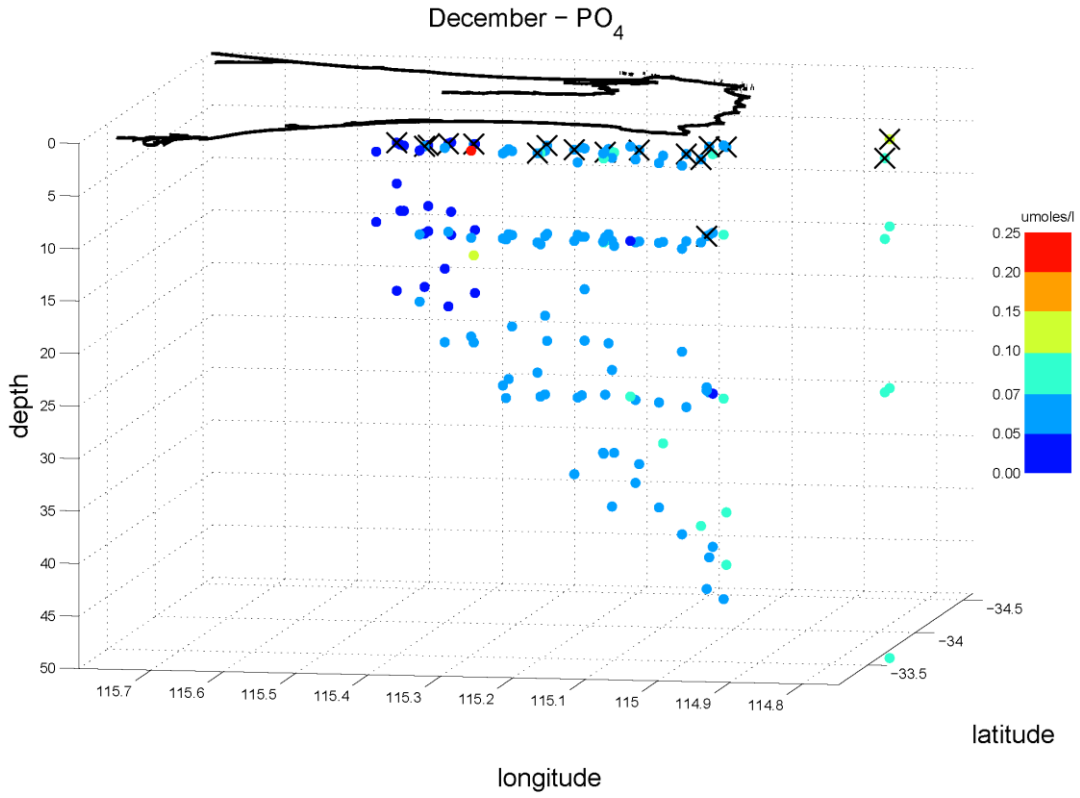
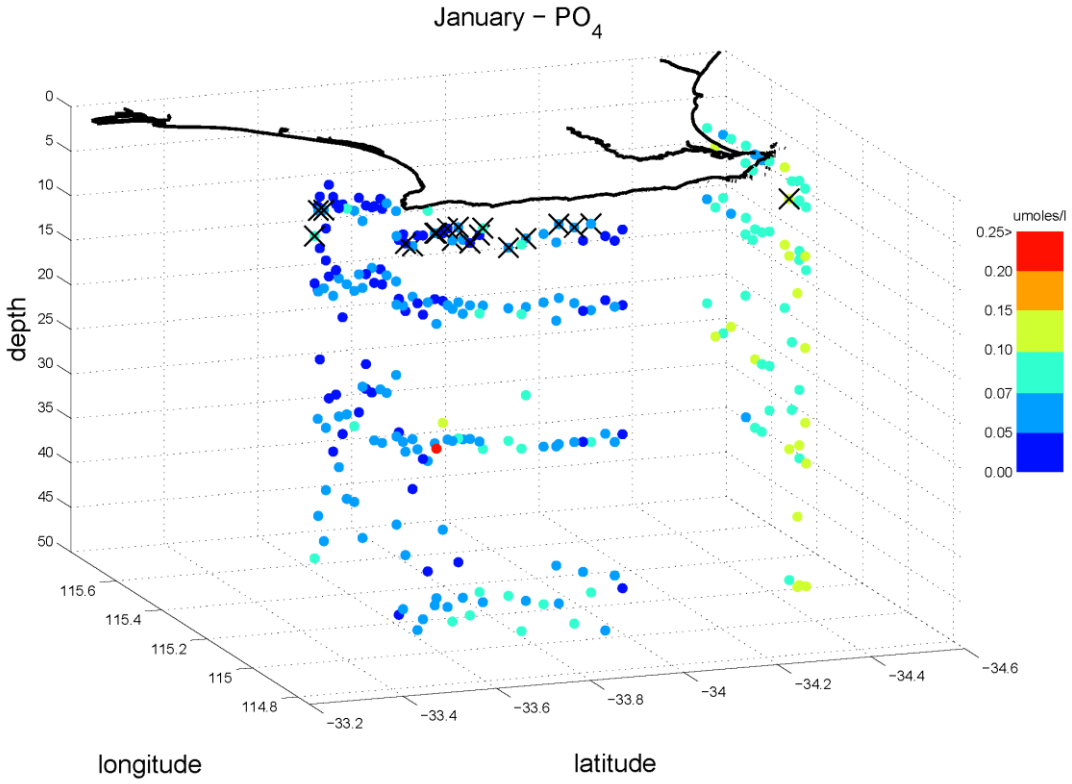


Figure 25 Concentration of nitrite/nitrate in the Capes area. Black crosses indicate stations positive for *G. hebraicum* and black contour indicates coastline.

Uniform phosphate concentrations were present in all 3 months with mean values of 0.05 (December and February) to 0.06 (January) µmol L^{-1} (Table 7). Minimum phosphate was in Geographe Bay and slightly higher concentrations were found north of Cape Leeuwin (Figure 26). The concentrations were lower than those found in Hanson et al (2005).





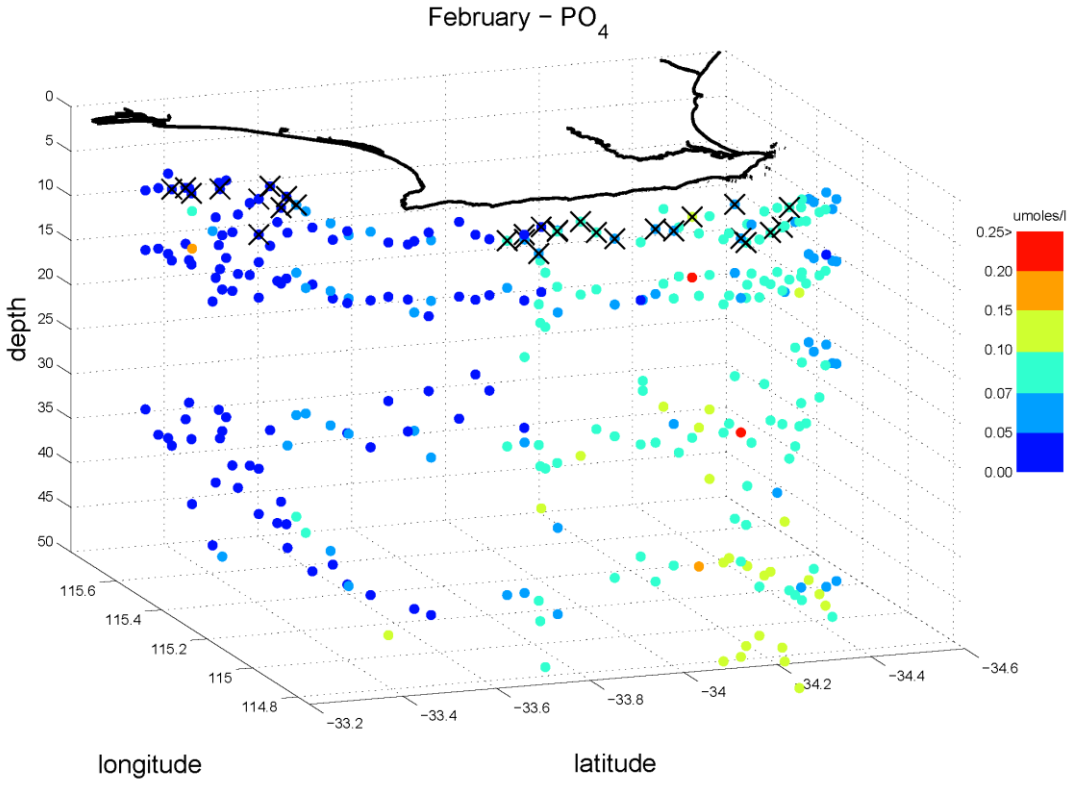
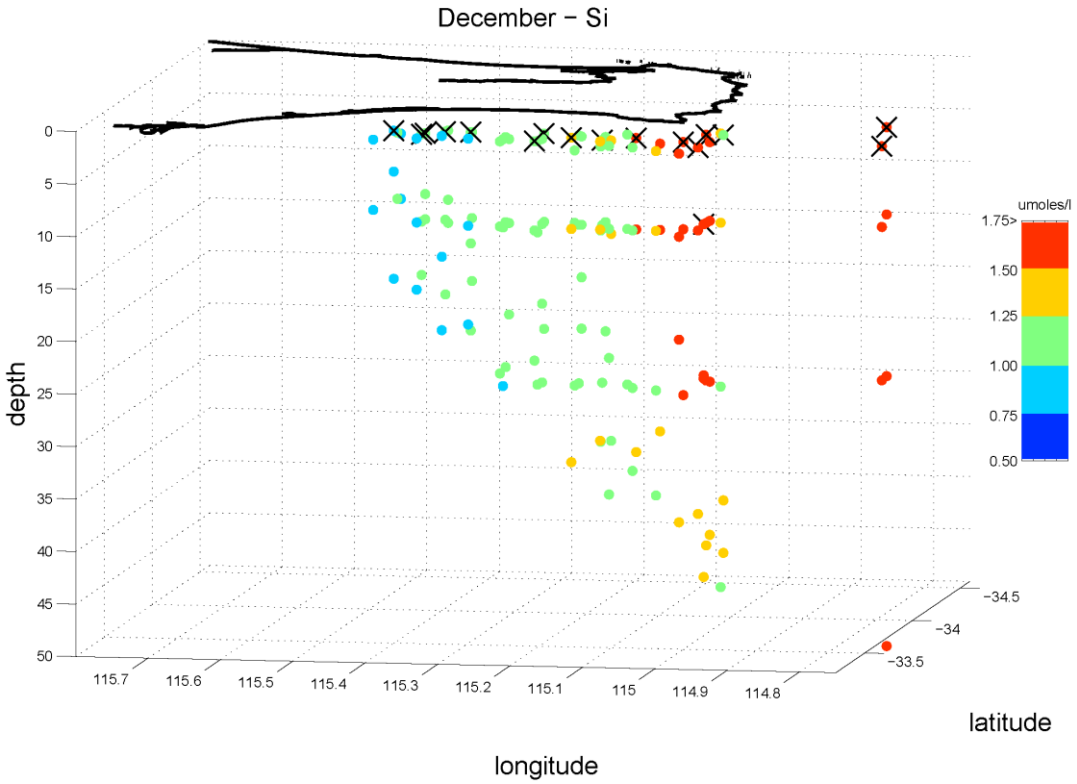


Figure 26 Concentration of phosphate in the Capes area. Black crosses indicate stations positive for *G. hebraicum* and black contour indicates coast outline.

Mean silica concentrations were 1.22 µmol L^{-1} in December and 1.11 L^{-1} in January and February (Table 7). Geographe Bay in February had a few high patches ranging from 1.25 to 1.75 µmol L^{-1}. Higher concentrations were also found off Cape Naturaliste in December and Cape Leeuwin in February (

Figure 27).



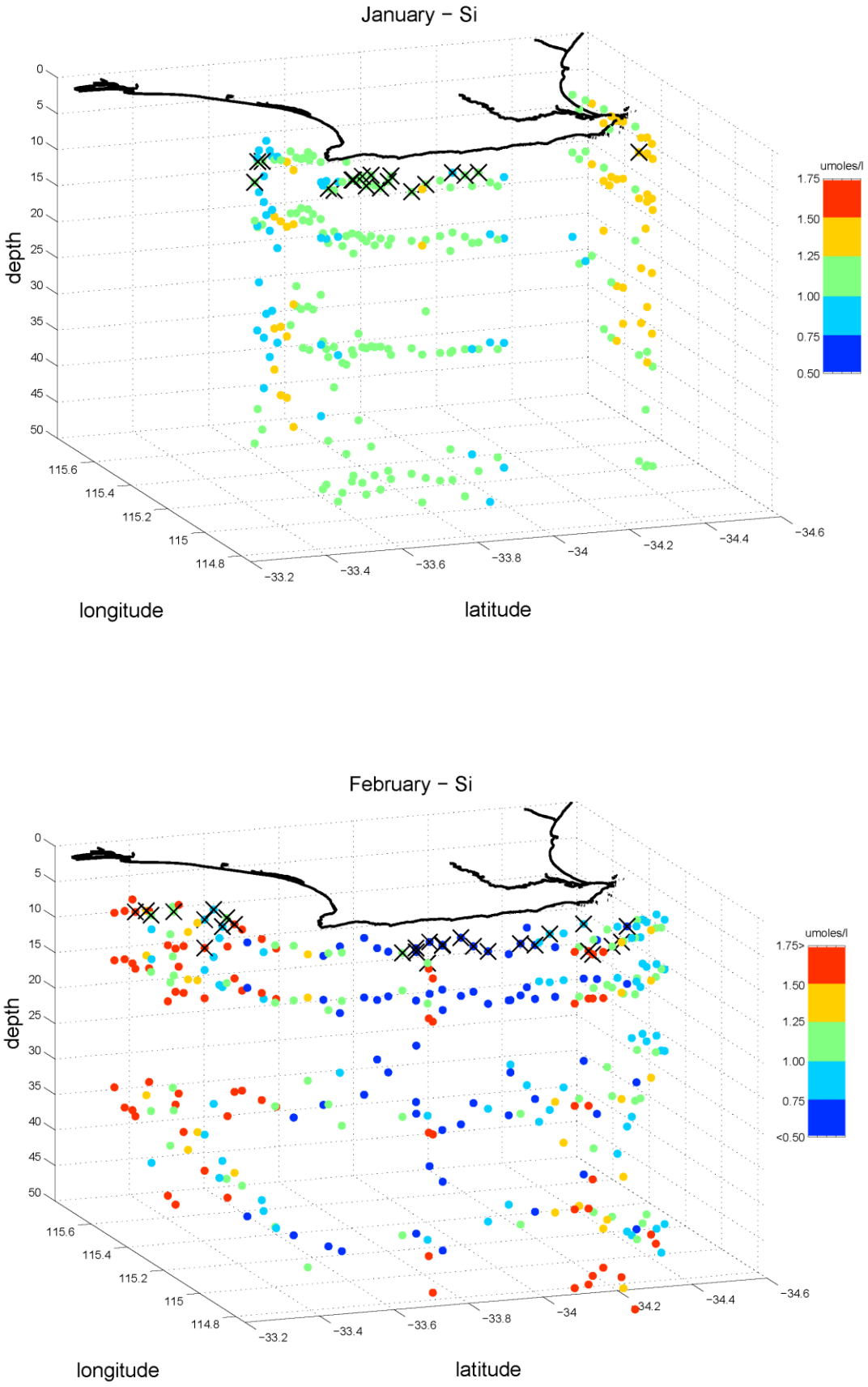
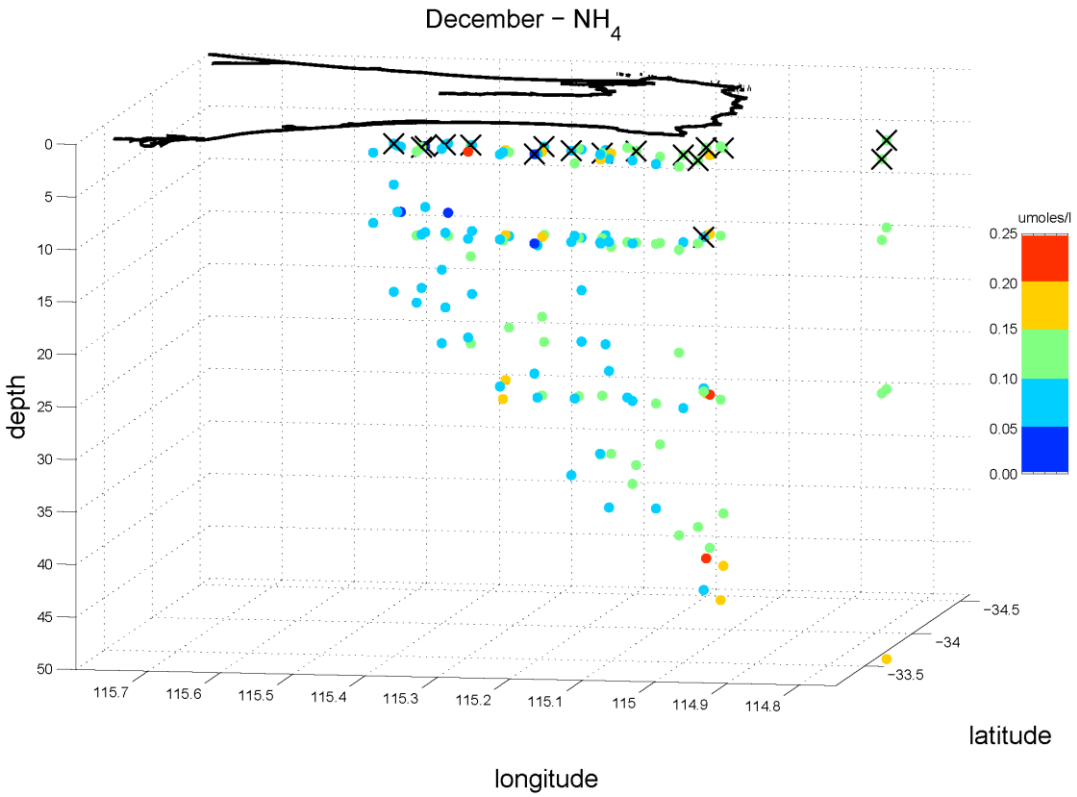


Figure 27 Concentration of silica in the Capes area. Black crosses indicate stations positive for *G. hebraicum* and black contour indicates coast outline.

Ammonium was generally low with double the concentration in December of 0.1 µmol L^{-1} compared with 0.06 and 0.07 µmol L^{-1} in January and February (Table 7). In all months there were a few isolated patches of elevated concentrations (0.2-0.25 µmol L^{-1}) (Figure 28).



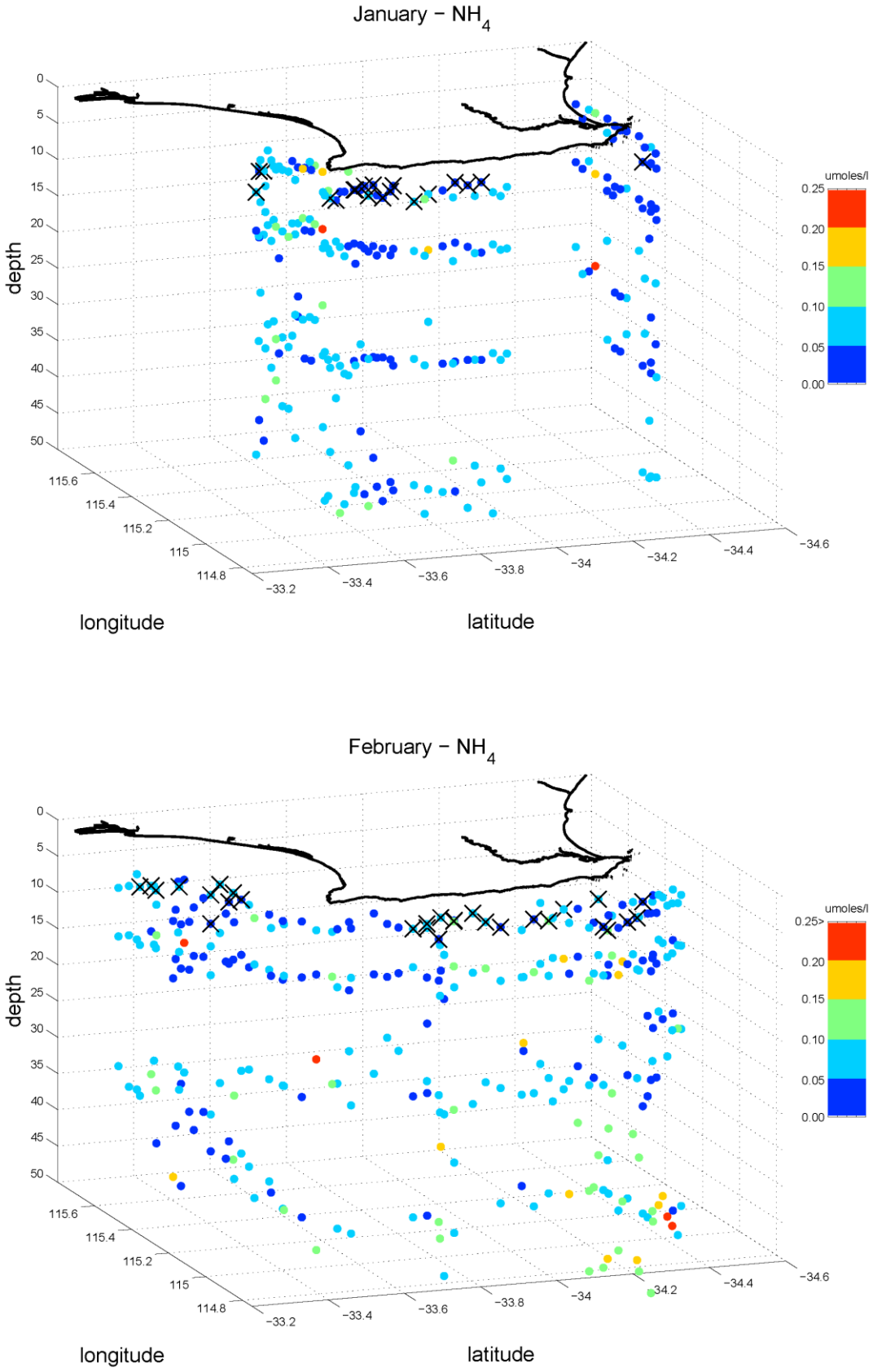
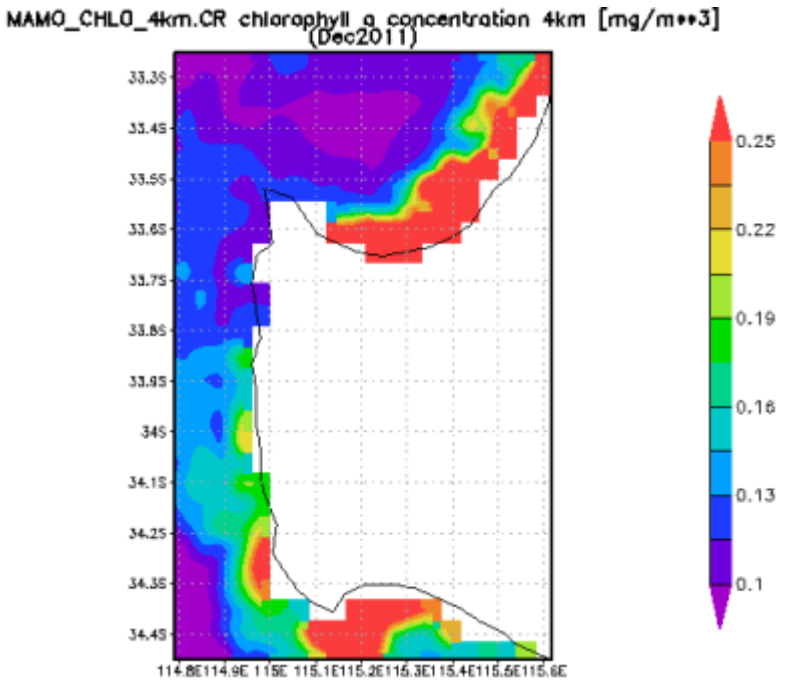
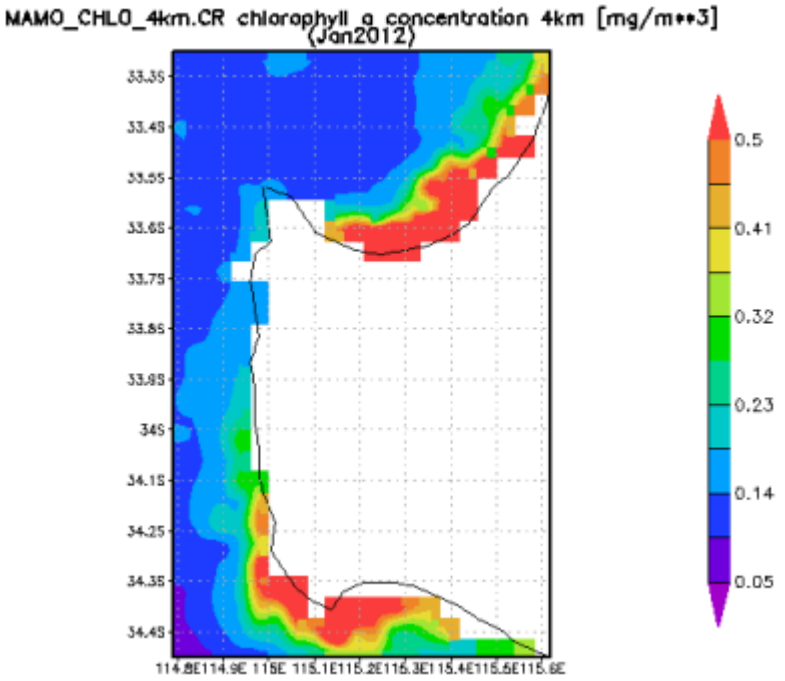


Figure 28 Concentration of ammonia in the Capes area. Black crosses indicate stations positive for *G. hebraicum* and black contour indicates coast outline.

The nitrate enrichment in February resulted in increased concentration of chlorophyll *a* in February which was evident both in ocean colour maps (Figure 29) and in extracted chlorophyll from water samples (Figure 30). Ocean colour maps showed patches of high chlorophyll in Geographe and Flinders Bay and around Cape Leeuwin with minima around Cape Naturaliste (Figure 29). Our samples showed increased chlorophyll at the surface and a chlorophyll maximum layer in February around Cape Leeuwin and less chlorophyll around Cape Naturaliste (Figure 30). In February there was significantly more chlorophyll both at the surface and in the chlorophyll maximum layer ($p= 0.000$ ANOVA $_{df=2,\ F=63.110}$ and $p=0.000$, ANOVA $_{df=2,\ F=105.823}$). There was also a higher proportion of larger phytoplankton (Table 7). The size of phytoplankton cells has been linked to nutrient availability in a previous study (Marañòn, et al, 2012). It is also known that phytoplankton size structure largely determines the trophic food chain and the efficiency of transfer to upper trophic levels i.e. fish larvae (Legendre and Rassoulzadegan, 1996).





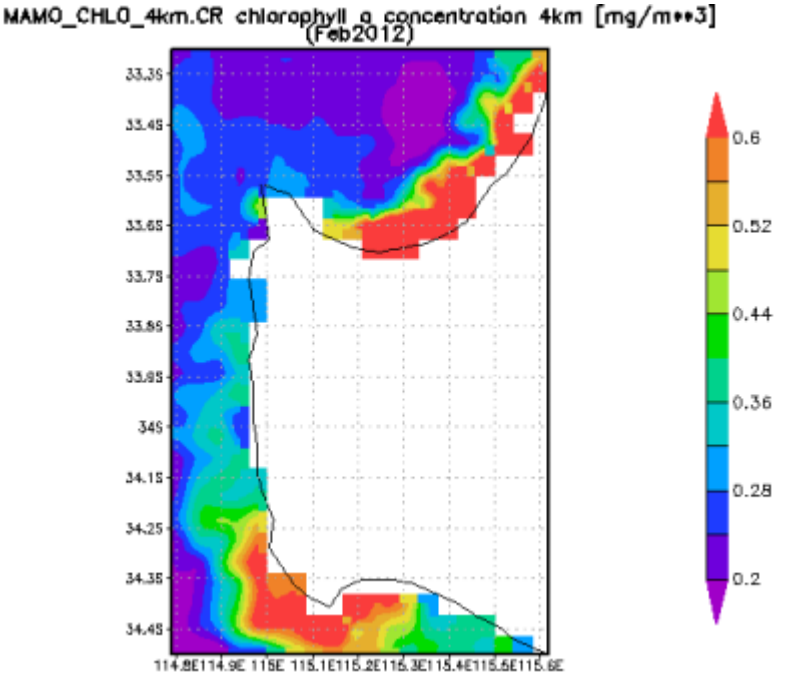
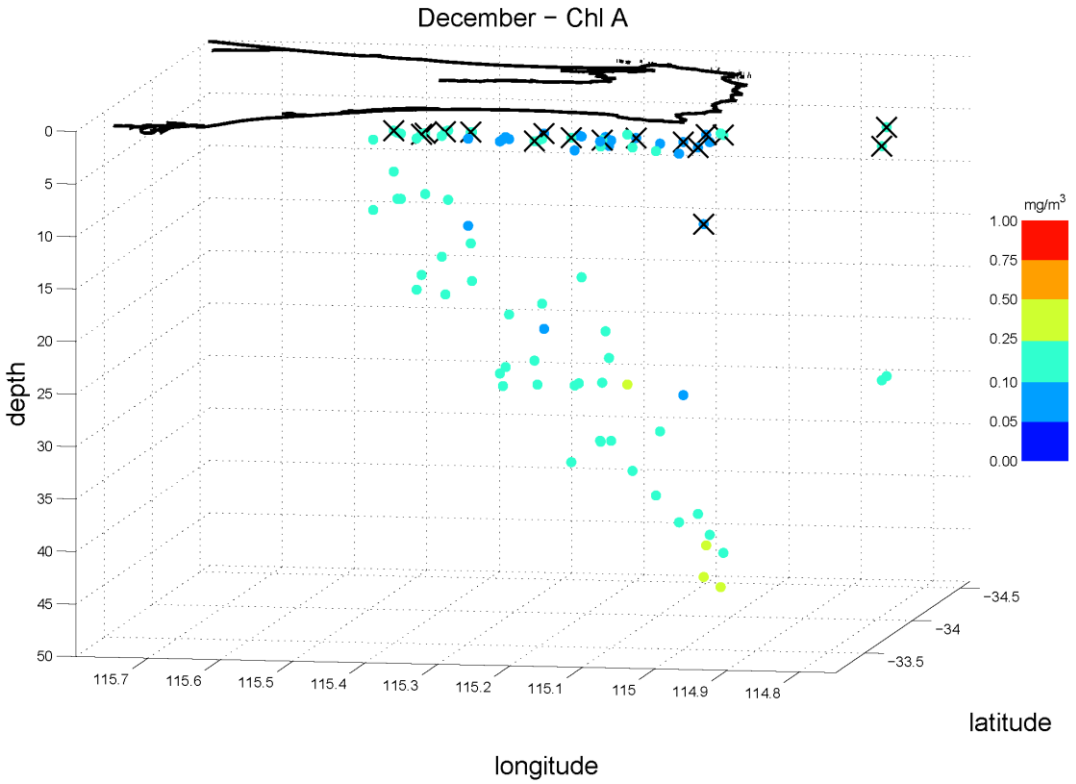
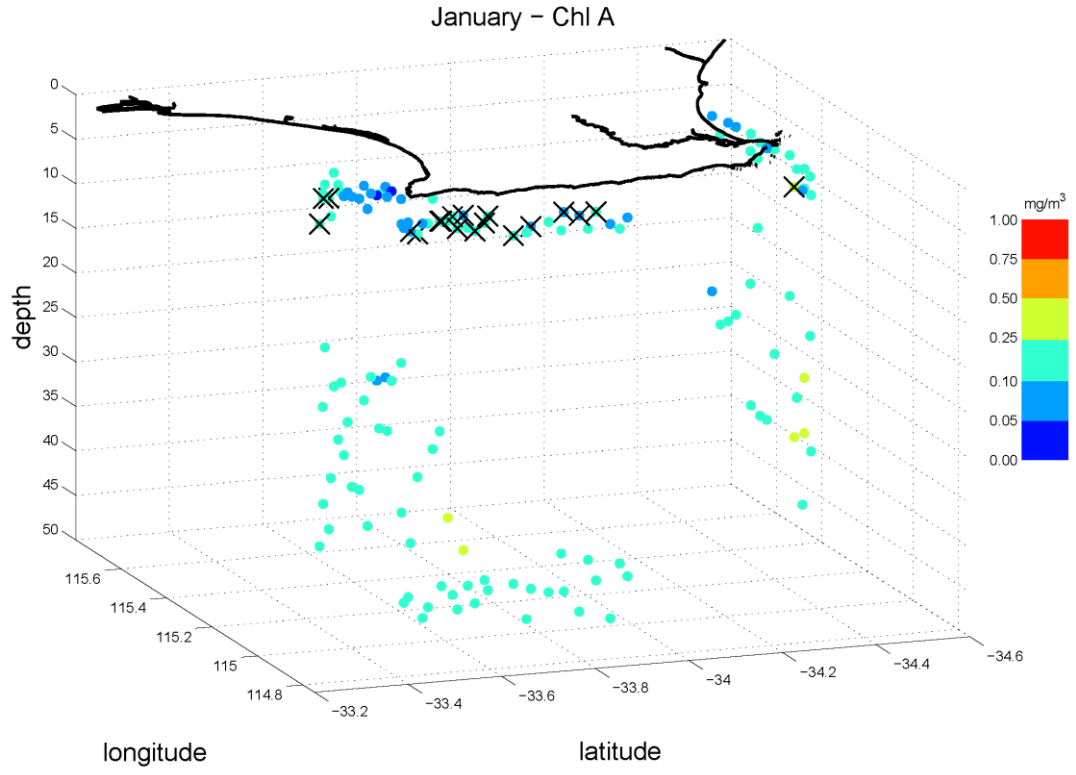


Figure 29 Ocean colour image of chlorophyll a around Capes area in three months (notice different chlorophyll scales).


December − Chl A
depth
0
5
10
15
20
25
30
35
40
45
50
115.7
115.6
115.5
115.4
115.3
115.2
115.1
115
114.9
114.8
longitude
−34.5
−34
−33.5
latitude
mg/m3
1.00
0.75
0.50
0.25
0.10
0.05
0.00


January − Chl A
depth
0
5
10
15
20
25
30
35
40
45
50
115.6
115.4
115.2
115
114.8
longitude
−33.2
−33.4
−33.6
−33.8
−34
−34.2
−34.4
−34.6
latitude
mg/m3
1.00
0.75
0.50
0.25
0.10
0.05
0.00

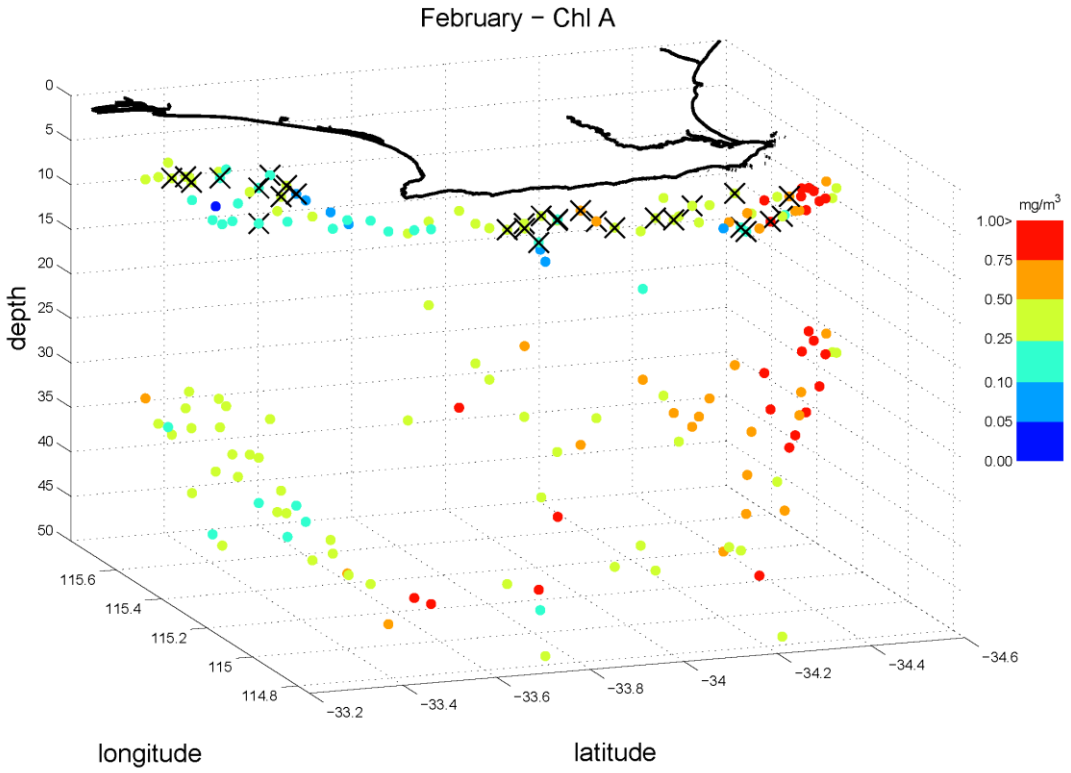


Figure 30 Concentration of total chlorophyll *a* in the Capes area. Black crosses indicate stations positive for *G. hebraicum* and black contour indicates coast outline.

parameter	December 2011	January 2012	February 2012
temperature (ºC)	20.73 (±0.16)	22.58 (±0.52)	22.98 (±0.20)
salinity (PSU)	35.45 (±0.04)	35.54 (±0.04)	35.53 (±0.08)
NO_2/NO_3 (µmol L^{-1})	0.01 (±0.01)	0.03 (±0.04)	0.11 (±0.14)
PO_4 (µmol L^{-1})	0.05 (±0.03)	0.06 (±0.03)	0.05 (±0.04)
SiO_3 (µmol L^{-1})	1.22 (±0.26)	1.11 (±0.14)	1.11 (±0.42)
NH_4 (µmol L^{-1})	0.10 (±0.03)	0.06 (±0.03)	0.07 (±0.02)
chlorophyll *a* surface (mg m^{-3})	0.12 (±0.05)	0.12 (±0.05)	0.36 (±0.22)
chlorophyll *a* maximum (mg m^{-3})	0.16 (±0.05)	0.18 (±0.05)	0.052 (±0.26)
% chl *a* > 5 µm	7.48 (±3.25)	6.42 ((±3.65)	15.27 (±7.15)

Table 7 Mean integrated temperature, salinity, and concentrations of nitrite/nitrate (NO_2/NO_3), phosphate (PO_4), silicate (SiO_3), ammonium (NH_4) and chlorophyll and the proportion of chlorophyll from large (>5 microns) phytoplankton (± standard deviations) in water samples from the Capes region from the three sampling trips.

We tested the correlation between total biomass and environmental variables from three months sampling in the Capes region to determine the importance of these factors on larvae. There was no

connection between biomass index and nutrients or chlorophyll however, there was a positive correlation with temperature and a negative correlation with salinity (Table 8). A positive relationship between temperature and strength of year classes in fish has been described in many populations (for example Loeng, 1989, Hidalgo et al, 2011) and it has been speculated that the prey abundance is higher in warmer water patches.

Effect	Coefficient	Standard Error	Std. Coefficient	Tolerance	t	p-value
temperature total	0.098	0.030	0.524	0.459	3.268	0.002
salinity total	-1.365	0.478	-0.616	0.254	-2.855	0.006
NOx total	0.120	0.302	0.073	0.349	0.397	0.693
PO4 total	-0.400	0.656	-0.097	0.473	-0.610	0.544
NH4 total	-0.065	0.102	-0.133	0.274	-0.641	0.524
SiO3 total	-0.405	0.799	-0.066	0.693	-0.507	0.614
chl a surface total	0.107	0.167	0.109	0.408	0.639	0.525
chl a maximum total	-0.158	0.139	-0.203	0.374	-1.141	0.258

Table 8 Results from ordinary least square regression showing regression coefficients and p values for biomass index and environmental variables.

8. BENEFITS AND ADOPTION

G. hebraicum is an iconic and endemic finfish in Western Australia and one of the most commercially and recreationally important species in the West Coast Bioregion. Yet, the only information on larval biology has been from aquaculture studies (FRDC project 95-095) as they have not previously been studied in the wild.

Direct beneficiaries of this research are commercial, recreational sectors that exploit the resource and the Department of Fisheries Western Australia which manages the resource. Managers from the Western Australian Department of Fisheries identified finding eggs and larvae of *G. hebraicum* as a priority for this study and this objective has been achieved. The methods used in the project proved highly successful in finding *G. hebraicum* larvae or eggs for the first time and this project provides baseline data on their location in the Capes region and the metropolitan area. The hydrodynamic modelling and drifters related the presence of early life stages to oceanographic conditions during sampling and predicted routes of larval transport relating it to currents and winds. The modelling showed that retention of larvae will depend on the larval behaviour and that vertical migration will potentially prevent larvae from being advected offshore away from suitable settlement areas. The drifters identified that the circulation in the Capes region is very complex and that there is an ongoing need for further measurements. The project provided more data on phytoplankton dynamics in the region. Phytoplankton is at the base of the food chain and the size

of the phytoplankton influences the marine food web. It is an important indication of food availability for fish larvae. In turn prey availability is considered a major factor affecting larval survival and juvenile recruitment rates (Anderson, 1988, Murphy et al, 2012).

The development of these methods opens a route to study larval behaviour and monitor larval dynamics, not only in West Australian *G. hebraicum*, but also other species of importance. Recruitment success in fish depends on processes influencing the larval stages (Cushing, 1990). Information on how the abundance of larvae correlates with the biomass of spawning stock and oceanography increases the ability to predict recruitment which contributes to better adjustment of fishing mortality leading to reduced risk of overfishing.

9. FURTHER DEVELOPMENT

This was a one year project to provide proof of concept and a test of methods to locate early life stages of *G. hebraicum* and form a baseline essential for long term studies. Longer term studies are required to address intra- and interannual variability of larval recruitment success and the oceanographic variables that may influence this.

Development of an increased understanding of the early life history of *G. hebraicum* and factors affecting recruitment include the following:

1) Improved interpretation of hydrodynamic advection of eggs and larvae from spawning grounds:

Because of the high variability in circulation and its importance for larval dispersal and survival (and hence subsequent recruitment), it is recommended that further current drifter studies be undertaken in conjunction with future surveys in this region. Superimposed on the traditional seasonal pattern of northwards flow along the inner continental shelf between about October and March (the Capes Current) are the highly variable current fluctuations in response to changes in the local wind field. There are also likely to be variations in the circulation at inter-annual scales (particularly associated with the *El Niño/La Niña* cycle) which would contribute towards the variations in *G. hebraicum* recruitment between years. Deployment of mooring gears equipped with an Acoustic Doppler Current Profiler (ADCP), a Conductivity-Temperature-Pressure-Fluorescence sensor, and thermistors for the summer field survey periods would allow monitoring the temporal variability of the Capes Current and related temperature variations, and the wind driven cross-shelf transport. Use of the high resolution ROMS (Regional Oceanographic Model System) model, developed during WAMSI, CSIRO IMOS project and phase 1 of the FRDC *G. hebraicum* project, would allow simulation of the upper ocean current, temperature, and salinity structures.
If the project is extended I believe sampling needs to consider not only the south-west and metro, but also the mid-west. Recruitment dynamics appear to vary from south to north. Strong and weak cohorts in age class data are much more distinct in the south-west than the mid-west. What drives

this? As the WCDSF in each of these areas can be managed separately and currently consist of different levels of fishing effort/mortality – there are different risks to stocks dependent on which area we're talking about.

2) Information on larval behaviour: where are the larvae and how their distribution varies with age

Particle modelling in this project identified the importance of larval behaviour for retention of larvae in suitable settlement areas. Study of larval behaviour requires depth-stratified sampling using multinet plankton net towing nets at: surface, chlorophyll maximum layer and bottom at both night and day to account for vertical migration and to determine the length of time spent in the plankton prior to settling in demersal habitats. The latter knowledge would allow more accurate modelling of likely dispersal of larvae along the coast. DNA tests developed in the present project would provide identification in real time of presence/absence of eggs and larvae. In addition we recommend use of quantitative PCR techniques to index the biomass of larval *G. hebraicum*. If a reliable index can be developed then it has the potential to inform future recruitment strength to the fished stocks. Next-generation DNA sequencing techniques could also be used to identify the larvae and eggs of other finfish species present at sampling sites.

3) Food for larvae: what do they eat and how does this relate to what is available (quality and quantity)

We recommend a combination of fatty acids and stable isotopes to identify long term (days to weeks) food sources. We also recommend trial of DNA-based approaches to describe current diet from digestive tract. This molecular method allows identifying food of even highly digested prey that have lost all the physical characteristics.

4) Do larvae show any association with particular types of habitats?

This project showed high correlation of eggs/larvae with a green alga *Cladophora* sp. Identification of association with benthic habitats would allow monitoring the health of habitats important for larvae. Video tows in conjunction with net sampling would characterise benthic habitats where larvae occur.

10. PLANNED OUTCOMES

The project has been a successful one year proof of concept and test of methods. It provided evidence for a combined modelling and genetics approach to guiding oceanographic sampling for eggs and larvae when there is little prior knowledge on biology of the species. The hydrodynamic model guided and optimised oceanographic sampling by identifying likely transport of larvae from suspected spawning locations. Genetics allowed rapid processing of collected plankton samples (12 samples in 2 hours) providing instantaneous feedback of success or failure of finding eggs or larvae of *G. hebraicum*. Shipboard detection allowed adaptive sampling maximising chances of success.

The project demonstrated how a particle (larvae) tracking model can provide information on early life history of *G. hebraicum*. Results showed that larvae in nearshore habitats can be rapidly carried offshore by surface Ekman flows unless they vertically migrate and then use onshore return flows to maintain their position on the shelf. The model indicated that there are retention areas around Capes Naturaliste and Leeuwin and in Geographe Bay.

The project showed that spawning location will play a crucial role in larval retention and offshore dispersion. The trajectories of drifters demonstrated the high level of current variability in areas of *G. hebraicum* spawning indicating that spawning time is crucial in larval survival to recruitment or loss from the population.

The project also brings new knowledge and new technology: a high resolution hydrodynamic model to guide sampling and a rapid and high -throughput DNA-based system to identify fish species from mixed zooplankton samples. These can be used in similar studies. As a proof of concept the project used quantitative PCR to measure the concentration of *G. hebraicum* DNA in mixed zooplankton samples providing a relative index of biomass.

The combination of techniques used in the project has an array of applications where there is little knowledge of the location of larvae or organisms are difficult to identify for example in the measurement of recruitment, monitoring the impacts of climate change on shifts of organisms or biosecurity.

The location and transport of eggs and larvae in relation to environmental characteristics like currents, salinity, temperature, nutrients and chlorophyll *a* will lead to better understanding of the biology of this fish and could lead to improved management of the fishery. Information on early life stages could allow predictions to be made about recruitment strength as advance information for the management of the fishery. Egg abundance could be also potentially used for spawning stock abundance estimate. *G hebraicum* is a primary target of boat-based line fishers in the West Coast Bioregion (WCB). The West Coast Demersal Scalefish Interim Managed Fishery comprising 60 permit holders reported catching 54 t of West Australian *G. hebraicum* in 2010 worth ca $900,000.

A further 16 t was landed by other fisheries in the bioregion (Fairclough et al., 2011). In 2009/10, 95 t of *G. hebraicum* was estimated to have been taken by the recreational and charter sectors in the WCB, which together are of significant value to the state in terms of economic value and employment. In 2009/10 125 charter vessels were licensed to operate in the WCB and 115,000 Recreational Fishing from Boat licenses had been issued up until March 2011 (Fairclough et al., 2011).

These numbers do not include social values such as lifestyle benefits. *G hebraicum* also has a high ecological value as a top predator and its loss from the ecosystem could disrupt trophic dynamics and biodiversity (Platell, 2010).

The project results were disseminated through workshops and conferences providing direct information to other scientists and stakeholders including at the 6th World Fisheries Congress in Edinburgh, Scotland (May, 2012), the Australian Marine Science Conference in Hobart (July 2012) and a seminar at the University of Western Australia (August 2012). Principal investigator and co-investigators presented the methods and preliminary results to fishery scientists, managers and fishers during a seminar in the Department of Fisheries in April 2012 and to WAFIC (May, 2012) and Recfishwest (August, 2012). A second seminar was given at the Department of Fisheries in Spetember to present final results of the project to stakeholders, including Recfishwest and WAFIC.

11. CONCLUSIONS

This project has provided a proof of concept of the methods employed. A combination of hydrodynamic modelling, field sampling and rapid genetic identification provided baseline data on the location of eggs and larvae of West Australian *G. hebraicum* in the Capes region of south-western Australia. This is the first step towards developing research into larval recruitment of this species. In particular, the project has demonstrated the advantage of using DNA techniques for identification of target taxa from mixed zooplankton samples to adapt sampling and maximise success rate. Furthermore, the quantitative PCR method tested here can be used as a relative index of biomass. This may be able to be developed to provide some form of recruitment index for WA *G. hebraicum* and thus as an early indicator of the expected future strength of individual cohorts as they recruit to the fishery. However, this would require ongoing monitoring of larval recruitment and the age structure of the fished stock(s) and would benefit from concomitant monitoring of juvenile *G. hebraicum* recruitment (see Mitsopoulos and Molony, 2010; Lewis et al., in prep.)

Particle tracking models shed light on early stage life history of *G. hebraicum*. Simulation can be used to indicate potential retention areas. Larval behaviour including temperature dependent

growth rates and vertical migration has potentially important consequences for larval retention and incorporating this behaviour in the model is likely to improve model predictions.

The drifter programme has confirmed the high variability of the currents along the continental shelf in the Capes region, with clear implications for larval transport of *G. hebraicum* and other commercial fish species. Improved knowledge of the nearshore circulation is needed to inform modelling. To achieve it, further deployments of drifters are recommended along with a mooring with an Acoustic Doppler Current Profiler (ADCP), a Conductivity-Temperature-Pressure-Fluorescence sensor, and thermistors. Because there are also likely to be variations in the circulation at inter-annual scales (particularly associated with the *El Niño/La Niña* cycle) which would contribute towards the variations in *G. hebraicum* recruitment between years a long term program of observations is recommended.

Another indicator variable that is likely to influence *G hebraicum* recruitment is growth and development in plankton. Lack of food can increase mortality either through starvation or slow growth and vulnerability to predation or disease. Phytoplankton observations from ocean colour data and from chlorophyll *a* biomass collected indicate variability in food availability for larvae. This is only a relative comparison that has limitations since larval nutrition depends on the prey field encountered by fish larvae. Analysis of biomarkers like fatty acids or stable isotopes from larvae is a more direct measurement of food quality and quantity available to larvae and is recommended for future study.

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12. APPENDIX 1: intellectual property

The research is for public domain. The report and resulting manuscripts are intended for wide dissemination and promotion.

13. APPENDIX 2: staff

The following table lists project staff

Name	Government organisation	Funding
John Keesing	CSIRO	FRDC and in-kind
Oliver Berry	CSIRO	FRDC and in-kind
Ming Feng	CSIRO	FRDC and in-kind
Liejun Zhong	CSIRO	FRDC and in-kind
Joanna Strzelecki	CSIRO	FRDC and in-kind
Dirk Slawinski	CSIRO	FRDC and in-kind
Douglas Bearham	CSIRO	FRDC and in-kind
James McLaughlin	CSIRO	FRDC and in-kind
Damian Thomson	CSIRO	FRDC and in-kind
David Fairclough	Department of Fisheries, Western Australia	in-kind
Gary Jackson	Department of Fisheries, Western Australia	in-kind
Alan Pearce	Department of Fisheries, Western Australia	in-kind
Jan Richardson	Department of Fisheries, Western Australia	in-kind
Greg Jenkins	Challenger Institute of Technology	in-kind
Gavin Partridge	Challenger Institute of Technology	in-kind
Francisco Neira	MARSCCO	FRDC

14. APPENDIX 3: station list

Date	Vessel	station id	latitude	longitude	station depth (m)
6/12/2011	Linnaeus	Li 1	33º44.09	114º45.044	60
6/12/2011	Linnaeus	Li 2	33^{0}39.57	114^{0}44.7412	60.5
7/12/2011	Linnaeus	Li 3	33º39.01	114º57.89	45
7/12/2011	Linnaeus	Li 4	33^{0}36.37	114^{0}58.04	48
7/12/2011	Linnaeus	Li 5	33º34.12	114º57.85	46
8/12/2011	Linnaeus	Li 6	33.56.63	114^{0}96.16	47
8/12/2011	Linnaeus	Li 7	33º31.84	115º04.91	33
9/12/2011	Linnaeus	Li 8	33^{0}31.98	115^{0}06.97	32
9/12/2011	Linnaeus	Li 9	33º31.94	115º08.92	30
9/12/2011	Linnaeus	Li 10	33^{0}32.00	115^{0}12.15	30
9/12/2011	Linnaeus	Li 11	33º31.89	115º15.12	27
10/12/2011	Linnaeus	Li 12	33^{0}29.95	115^{0}07.88	35
10/12/2011	Linnaeus	Li 13	33º29.99	115º10.11	34
10/12/2011	Linnaeus	Li 14	33^{0}29.98	115^{0}11.78	33
10/12/2011	Linnaeus	Li 15	33º29.95	115º14.90	31
11/12/2011	Linnaeus	Li 16	33^{0}34.00	115.14.85	23
11/12/2011	Linnaeus	Li 17	33º34.00	115º11.87	25
11/12/2011	Linnaeus	Li 18	33.33.99	115^{0}09.55	26
11/12/2011	Linnaeus	Li 19	33º33.91	115º06.93	25
11/12/2011	Linnaeus	Li 20	33^{0}29.87	115^{0}17.94	27
12/12/2011	Linnaeus	Li 21	33º30.07	115º24.20	25.6
12/12/2011	Linnaeus	Li 22	33^{0}10.17	118^{0}21.88	24
12/12/2011	Linnaeus	Li 23	33º29.97	115º21.70	19
12/12/2011	Linnaeus	Li 24	33^{0}31.90	115^{0}18.03	24
13/12/2011	Linnaeus	Li 25	33º35.97	115º23.88	12
13/12/2011	Linnaeus	Li 26	33^{0}33.78	115^{0}21.97	14
13/12/2011	Linnaeus	Li 27	33º33.94	115º20.03	16.7
14/12/2011	Linnaeus	Li 28	33^{0}33.83	115^{0}17.76	22
14/12/2011	Linnaeus	Li 29	33º31.54	115º20.34	22
14/12/2011	Linnaeus	Li 30	33^{0}31.99	115^{0}22.00	19
14/12/2011	Linnaeus	Li 31	33º32.38	115º25.61	14
14/12/2011	Linnaeus	Li 32	33^{0}32.37	115^{0}26.72	10
15/12/2011	Linnaeus	Li 33	33º27.23	115º16.98	33
15/12/2011	Linnaeus	Li 34	33^{0}28.02	115^{0}11.75	35
15/12/2011	Linnaeus	Li 35	32º28.06	115º08.90	37
15/12/2011	Linnaeus	Li 36	33^{0}27.84	115^{0}06.60	39

16/12/2011	Linnaeus	Li 37	33º26.17	115º04.31	39
16/12/2011	Linnaeus	Li 38	33^{0}28.07	115^{0}03.97	43
16/12/2011	Linnaeus	Li 39	33.26.94	115º02.87	46
16/12/2011	Linnaeus	Li 40	33^{0}29.14	115^{0}01.07	47
17/12/2011	Linnaeus	Li 41	33º31.29	115º08.42	36
17/12/2011	Linnaeus	Li 42	33^{0}31.29	115^{0}08.42	39
17/12/2011	Linnaeus	Li 43	33º29.71	115º02.31	39.9
17/12/2011	Linnaeus	Li 44	33^{0}28.98	114^{0}59.45	36
18/12/2011	Linnaeus	Li 45	33º31.84	114º58.93	41

Date	**Vessel**	**station id**	**latitude**	**longitude**	**station depth (m)**
17/01/2012	Naturalist	Na 1	33º33.36	115º06.74	24
17/01/2012	Naturalist	Na 2	33^{0}33.65	115^{0}09.28	26.1
17/01/2012	Naturalist	Na 3	33º32.72	115º10.94	26.1
17/01/2012	Naturalist	Na 4	33^{0}33.60	115^{0}12.85	25.2
18/01/2012	Naturalist	Na 5	33º28.10	115º04.18	41.7
18/01/2012	Naturalist	Na 6	33^{0}27.94	115^{0}05.84	38.6
18/01/2012	Naturalist	Na 7	33º28.00	115º07.76	38
18/01/2012	Naturalist	Na 8	33^{0}28.00	115^{0}92.40	36.2
20/01/2012	Naturalist	Na 9	34º23.94	115º22.12	31.2
20/01/2012	Naturalist	Na 10	34^{0}23.98	115^{0}79.23	32
20/01/2012	Naturalist	Na 11	34º24.00	115º25.94	31.1
20/01/2012	Naturalist	Na 12	34^{0}24.02	115^{0}27.52	23.2
21/01/2012	Naturalist	Na 13	34º24.08	115º20.06	34.1
21/01/2012	Naturalist	Na 14	34^{0}24.98	115^{0}18.44	34.4
21/01/2012	Naturalist	Na 15	34º26.09	115º17.23	35.3
21/01/2012	Naturalist	Na 16	34^{0}24.94	115^{0}15.80	35.9
21/01/2012	Naturalist	Na 17	34º21.85	115º12.08	14.7
21/01/2012	Naturalist	Na 18	34^{0}21.81	115^{0}13.57	16.9
22/01/2012	Naturalist	Na 19	34º25.07	115º10.99	24.4
22/01/2012	Naturalist	Na 20	34^{0}25.11	115^{0}08.52	29.9
22/01/2012	Naturalist	Na 21	34º25.04	115º07.26	27.4
22/01/2012	Naturalist	Na 22	34^{0}24.88	115^{0}05.72	36.3
22/01/2012	Naturalist	Na 23	34º22.09	115º00.00	47.3
22/01/2012	Naturalist	Na 24	34^{0}30.24	114^{0}56.91	46
22/01/2012	Naturalist	Na 25	34º21.01	114º58.07	46.4
22/01/2012	Naturalist	Na 26	34^{0}20.86	114^{0}28.58.	47
23/01/2012	Naturalist	Na 27	33º31.99	115º06.96	30.5
23/01/2012	Naturalist	Na 28	33^{0}31.99	115^{0}08.69	29.7

23/01/2012	Naturalist	Na 29	33º32.00	115º09.98	29.5
23/01/2012	Naturalist	Na 30	33^{0}32.03	115^{0}11.58	27.7
23/01/2012	Naturalist	Na 31	33º30.33	115º13.03	30.1
24/01/2012	Naturalist	Na 32	33^{0}31.95	114^{0}58.89	41.7
24/01/2012	Naturalist	Na 33	33º33.54	115º904.5	44.1
24/01/2012	Naturalist	Na 34	33^{0}34.43	114^{0}58.89	44.2
24/01/2012	Naturalist	Na 35	33º36.19	114º58.92	43
24/01/2012	Naturalist	Na 36	33^{0}34.17	114.57.03	45.7
24/01/2012	Naturalist	Na 37	33º35.37	114º58.02	46.4
25/01/2012	Naturalist	Na 38	33^{0}37.99	114^{0}57.94	44.5
25/01/2012	Naturalist	Na 39	33º39.36	114º57.90	43.1
25/01/2012	Naturalist	Na 40	34^{0}40.11	114^{0}56.92	44.1
25/01/2012	Naturalist	Na 41	33º39.80	114º56.58	44
25/01/2012	Naturalist	Na 42	33^{0}37.08	114^{0}56.60	45
25/01/2012	Naturalist	Na 43	33º35.25	114º56.71	46.5
26/01/2012	Naturalist	Na 44	33^{0}31.81	114^{0}56.50	48
26/01/2012	Naturalist	Na 45	33º30.57	114.56.55	50.6

Date	**Vessel**	**station id**	**latitude**	**longitude**	**station depth (m)**
15/02/2012	Naturalist	Na 71	33º25.35	114º13.21	36.8
15/02/2012	Naturalist	Na 72	33^{0}23.34	114^{0}11.73	40.1
15/02/2012	Naturalist	Na 73	33º25.08	114º08.25	40.4
15/02/2012	Naturalist	Na 74	33^{0}25.87	114^{0}05.13	41.6
15/02/2012	Naturalist	Na 75	33º28.05	114º01.88	45.6
15/02/2012	Naturalist	Na 76	33^{0}30.60	114^{0}59.23	49.2
15/02/2012	Naturalist	Na 77	33º32.95	114º58.00	46.5
16/02/2012	Naturalist	Na 78	33^{0}42.31	114^{0}55.01	43.2
16/02/2012	Naturalist	Na 79	33º43.64	114º54.90	43.1
16/02/2012	Naturalist	Na 80	33^{0}44.39	114^{0}54.95	43.4
16/02/2012	Naturalist	Na 81	33º47.00	114º54.87	45.3
16/02/2012	Naturalist	Na 82	33^{0}41.43	114^{0}47.77	43.4
16/02/2012	Naturalist	Na 83	33º42.50	114º45.88	47.6
17/02/2012	Naturalist	Na 84	33^{0}54.70	114^{0}52.22	41.6
17/02/2012	Naturalist	Na 85	33º56.77	114º52.72	40.3
17/02/2012	Naturalist	Na 86	33^{0}59.33	114^{0}53.23	41.6
17/02/2012	Naturalist	Na 87	34º01.48	114º53.25	41.4
17/02/2012	Naturalist	Na 88	34^{0}03.31	114^{0}53.59	41.5
17/02/2012	Naturalist	Na 89	34º04.78	114º53.83	41.6
17/02/2012	Naturalist	Na 90	34^{0}07.09	114^{0}48.92	50.1

17/02/2012	Naturalist	Na 91	34º08.30	114º49.15	49.9
18/02/2012	Naturalist	Na 92	34^{0}10.08	114^{0}53.83	41.2
18/02/2012	Naturalist	Na 93	34º11.55	114º54.11	42
18/02/2012	Naturalist	Na 94	34^{0}12.97	114^{0}54.34	43.5
18/02/2012	Naturalist	Na 95	34º14.21	114º54.84	43.1
18/02/2012	Naturalist	Na 96	34^{0}15.23	114^{0}55.14	43.6
18/02/2012	Naturalist	Na 97	34º16.30	114º52.43	49.2
18/02/2012	Naturalist	Na 98	34^{0}14.30	114^{0}51.58	47.4
18/02/2012	Naturalist	Na 99	34º12.24	114º50.71	48.1
19/02/2012	Naturalist	Na 100	34^{0}16.08	114^{0}52.20	49.6
19/02/2012	Naturalist	Na 101	34º17.71	114º52.77	50.9
19/02/2012	Naturalist	Na 102	34^{0}18.80	114^{0}53.01	60.7
19/02/2012	Naturalist	Na 103	34º18.17	114º56.85	46.3
19/02/2012	Naturalist	Na 104	34^{0}19.83	114^{0}57.64	47.2
19/02/2012	Naturalist	Na 105	34º20.87	114º58.30	47.3
19/02/2012	Naturalist	Na 106	34^{0}21.71	114^{0}58.87	46.7
20/02/2012	Naturalist	Na 107	34º26.61	114º03.55	47.7
20/02/2012	Naturalist	Na 108	34^{0}26.86	114^{0}03.39	48.1
20/02/2012	Naturalist	Na 109	34º25.54	115º03.56	46.1
20/02/2012	Naturalist	Na 110	34^{0}27.53	115^{0}01.49	48.1
20/02/2012	Naturalist	Na 111	34º25.97	114º59.64	45.4
20/02/2012	Naturalist	Na 112	34^{0}24.51	114^{0}58.30	45.8
20/02/2012	Naturalist	Na 113	34º23.14	114º57.20	45.7
21/02/2012	Naturalist	Na 114	34^{0}18.36	114^{0}58.92	31.6
21/02/2012	Naturalist	Na 115	34º16.67	114º58.81	36.3
21/02/2012	Naturalist	Na 116	34^{0}14.65	114^{0}59.11	36.6
21/02/2012	Naturalist	Na 117	34º11.46	114º58.19	34.1
21/02/2012	Naturalist	Na 118	34^{0}09.44	114^{0}59.64	28.8
21/02/2012	Naturalist	Na 119	34º06.88	114º58.07	30.1
21/02/2012	Naturalist	Na 120	34.05.03	114^{0}58.83	29.6
21/02/2012	Naturalist	Na 121	34º02.71	114º58.16	29
21/02/2012	Naturalist	Na 122	33^{0}59.72	114^{0}58.03	32.6
22/02/2012	Naturalist	Na 123	33º54.89	114º57.88	25.3
22/02/2012	Naturalist	Na 124	33^{0}53.01	114^{0}58.27	29.2
22/02/2012	Naturalist	Na 125	33º49.58	114º57.21	24.1
22/02/2012	Naturalist	Na 126	33^{0}48.32	114^{0}59.08	31
22/02/2012	Naturalist	Na 127	33º46.01	114º58.24	30.6
22/02/2012	Naturalist	Na 128	33^{0}41.56	114^{0}58.42	20.6
22/02/2012	Naturalist	Na 129	33º40.01	114º59.18	28.4
22/02/2012	Naturalist	Na 130	33^{0}38.54	114^{0}00.54	29.7

23/02/2012	Naturalist	Na 131	33º37.64	114º01.01	21.90
23/02/2012	Naturalist	Na 132	33^{0}35.82	114^{0}00.65	21.20
23/02/2012	Naturalist	Na 133	33º34.40	114º00.03	17.80
23/02/2012	Naturalist	Na 134	33^{0}33.15	114^{0}59.87	30.70
23/02/2012	Naturalist	Na 135	33º28.89	114º58.99	48.70
23/02/2012	Naturalist	Na 136	33^{0}27.97	114^{0}00.46	48.50
23/02/2012	Naturalist	Na 137	33º26.52	114º01.80	43.60
23/02/2012	Naturalist	Na 138	33^{0}25.28	114^{0}03.01	43.70
24/02/2012	Naturalist	Na 139	33º22.21	114º05.56	41.80
24/02/2012	Naturalist	Na 140	33^{0}20.82	114^{0}05.76	40.70
24/02/2012	Naturalist	Na 141	33.19.06	114º05.98	40.2
24/02/2012	Naturalist	Na 142	33^{0}17.39	114^{0}06.05	38.20
24/02/2012	Naturalist	Na 143	33º15.67	114º06.36	34.30
24/02/2012	Naturalist	Na 144	33^{0}14.11	114^{0}06.36	35.40
24/02/2012	Naturalist	Na 145	33º11.41	114º09.31	41.70
24/02/2012	Naturalist	Na 146	33.12.90	114^{0}09.34	36.00
25/02/2012	Naturalist	Na 147	33º19.72	114º09.43	38.30
25/02/2012	Naturalist	Na 148	33^{0}20.56	114^{0}10.59	38.80
25/02/2012	Naturalist	Na 149	33º21.45	114º11.88	37.80
25/02/2012	Naturalist	Na 150	33^{0}22.59	114^{0}13.15	39.70
25/02/2012	Naturalist	Na 151	33º23.36	114º14.53	37.60
25/02/2012	Naturalist	Na 152	33^{0}24.23	114^{0}16.74	35.50
25/02/2012	Naturalist	Na 153	33º21.57	114º18.47	33.30
25/02/2012	Naturalist	Na 154	33^{0}20.20	114^{0}20.60	31.80
26/02/2012	Naturalist	Na 155	33º12.62	114º23.57	30.80
26/02/2012	Naturalist	Na 156	33^{0}14.10	114^{0}23.95	29.90
26/02/2012	Naturalist	Na 157	33º15.36	114º23.28	28.80
26/02/2012	Naturalist	Na 158	33^{0}16.50	114^{0}22.79	29.40
26/02/2012	Naturalist	Na 159	33º19.10	114º23.88	28.00
26/02/2012	Naturalist	Na 160	33^{0}17.45	114^{0}24.72	28.10
26/02/2012	Naturalist	Na 161	33º15.93	114º26.00	29.80
26/02/2012	Naturalist	Na 162	33^{0}14.34	114^{0}27.53	29.10
26/02/2012	Naturalist	Na 163	33º12.75	114º26.63	29.20

Date	**Vessel**	**station id**	**latitude**	**longitude**	**station depth (m)**
14/03/2012	Linnaeus	P1	31º52.89	115º39.43	28.00
14/03/2012	Linnaeus	P2	31º53.09	115º38.07	29.20
14/03/2012	Linnaeus	P3	31º53.77	115º38.17	30.60
14/03/2012	Linnaeus	P4	31º52.74	115º38.77	30.60

14/03/2012	Linnaeus	P5	31º59.68	115º38.71	29.70
15/03/2012	Linnaeus	P6	31º52.89	115º38.47	29.60
15/03/2012	Linnaeus	P7	31º53.20	115º38.64	29.60
15/03/2012	Linnaeus	P8	31º53.08	115º38.61	29.20
15/03/2012	Linnaeus	P9	31º52.81	115º38.59	28.90
15/03/2012	Linnaeus	P10	31º53.56	115º40.15	25.50

Date	Vessel	station id	latitude	longitude	station depth (m)
12/04/2012	Linnaeus	P11	31º52.89	115º39.43	28.00
12/04/2012	Linnaeus	P12	31º53.09	115º38.07	29.20
12/04/2012	Linnaeus	P13	31º53.77	115º38.17	30.60
12/04/2012	Linnaeus	P14	31º52.74	115º38.77	30.60
12/04/2012	Linnaeus	P15	31º59.68	115º38.71	29.70
13/04/2012	Linnaeus	P16	31º52.89	115º38.47	29.60
13/04/2012	Linnaeus	P17	31º53.20	115º38.64	29.60
13/04/2012	Linnaeus	P18	31º53.08	115º38.61	29.20
13/04/2012	Linnaeus	P19	31º52.81	115º38.59	28.90
13/04/2012	Linnaeus	P20	31º53.56	115º40.15	25.50

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30 May 2013
Our Ref: 2011/016 "Location and transport of early life stages of Dhufish (*Glaucosoma hebraicum*)"

Mr Crispian Ashby
FRDC Program Manager
Fisheries Research and Development Corporation
Locked Bag 222, Deakin West ACT 2600
Fisheries Research House, 25 Geils Court, Deakin

Dear Mr Ashby

Please find included five bound copies, one un-bound copy and an electronic version on CD of non-technical summary and the whole report in pdf and word format of the report for the project 2011/016 "Location and transport of early life stages of Dhufish (*Glaucosoma hebraicum*)".

Yours sincerely,

John Keesing
Research Scientist
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08 9333 6500

Government of Western Australia
Department of Fisheries