Ichthyoplankton Sampling Program:

December 2018 to December 2019

Technical Report A - Annexure G

AGL Gas Import Jetty Project

CEE Technical Report
Ichthyoplankton Sampling Program
Lower North Arm, Western Port

December 2018 to December 2019

February 2020

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Larval fish sample from Lower North Arm Sampling Program (CEE)

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[redacted]

Principal
AECOM

Report prepared by:
Scott Chidgey, Peter Crockett, Amelie Mendrinna, Merric Northey, Joana Costa
CEE Pty Ltd
Unit 4, 150 Chesterville Rd
Cheltenham, VIC 3192
cee.com.au

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1 Background

AGL Wholesale Gas Pty Ltd (AGL) and APA jointly propose to develop a Liquid Natural Gas (LNG) import terminal at Crib Point in the Lower North Arm of Western Port in Victoria Australia. They propose to moor a floating storage and regasification unit (FSRU) at Crib Point Jetty Berth 2, install gas offloading facilities on the jetty and construct a transfer pipeline between Crib Pt and Pakenham. AGL proposes to engage a contractor to supply and operate the FSRU facility, while APA will develop and operate the gas transfer pipeline.

The Victorian Minister for Planning (the Minister) decided that based on the referral documentation that was lodged on the project late in 2018 that an Environment Effects Statement under the *Environment Effects Act 1978* was required. The Victorian EES process is an accredited assessment process under the EPBC Act. The Minister issued scoping requirements (Ministerial Guidelines) for the EES in February 2018. The Ministerial Guidelines along with the EES referral documents were used to design technical studies that fulfil the requirements for the Environment Effects Statement.

The EES referral stage documents identified the pathways that project processes may impact upon the Western Port marine ecosystem. A key impact pathway in Western Port was entrainment of plankton in the seawater used in heat exchangers and other processes onboard the FSRU. One of the recommendations included in the EES referral was that an intensive plankton sampling program be developed to provide information on spatial and temporal variations in plankton populations in lower north arm focussing on the proposed location and position of the FSRU intake.

One of the recommendations included in the EES referral was that a plankton sampling program be developed to provide information on spatial and temporal variations in plankton populations in Lower North Arm focussing on the proposed location and position of the FSRU intake. This information would be used to inform the evaluation of *"potential for significant short and long-term impacts on marine biota due to entrainment of organisms in seawater for regasification or due to discharge of cooled seawater after use for regasification, including impacts resulting from reduced availability of food for other species, resultant hydrodynamic changes and other impacts such as long-term changes to populations and distribution".*

This technical report presents the rationale for the approach to the plankton studies and the methods used to sample, sort and identify the fish eggs and larvae in Lower North Arm. Results of ichthyoplankton (fish eggs and larvae) are presented with summary explanations.

This report is one of three reports on the plankton, the others being the zooplankton (macroinvertebrates) and phytoplankton. Further discussion of the results in the context of hydrodynamic, entrainment and discharge modelling, ichthyoplankton ecology, environmental guideline values and impact assessment are provided in CEEs head report to the EES (CEE 2020).

2 Background to ichthyoplankton in Western Port

The marine environment of Western Port is characterised by a range of habitats, including seagrass, mangroves, algae and reefs, that support a rich and diverse fish fauna (CEE 2009, Edgar and Shaw 1995, Jenkins *et al.* 2015, Robertson 1978). A range of fish species from these diverse habitats release their eggs and larvae into the water column where they mix and are dispersed by currents until they reach the early juvenile stage. The egg and larval stage in marine fish (ichthyoplankton) is characterised by high mortality rates, and the early stages are important in determining levels of recruitment and abundance of the population (Houde 2008). Spatial and temporal variability in Western Port ichthyoplankton communities are the product of life history (spawning location, spawning period, larval period), mortality (mainly predation), and dispersal related to the wind driven hydrodynamics in Bass Strait and tide dominated hydrodynamics in Western Port.

There has been commercial fishing in Western Port since the early 1900's (or earlier, Bennett 2004, Conron *et al.* 2016). In December 2007, commercial netting methods were no longer allowed in Western Port, so that since then there has only been a very low level of commercial fishing (Conron *et al.* 2016). At the same time, the recreational fishery in Western Port is very important and represents the second-largest recreational fishery in Victoria (Conron *et al.* 2016, Worsterling 2007). Although some key recreational fish caught in Western Port, such as snapper and King George whiting, do not breed in the bay, juveniles use the bay as a nursery area. In addition to species of importance to fishing, there are also conservation listed fish species such as pipefish in Western Port, as well as ecologically important species such as smooth toad fish and box fish that are key components of the food chain (Jenkins 2011). One of the key criteria for Western Port's listing as a Ramsar area is its importance as a "source of food for fishes, spawning ground, nursery and or migration path" (Criterion 8, KBR 2010).

In recognition of the gap in information on plankton communities in North Arm and the potential effects of the operation of a FSRU at Crib Point and the potential effects of entrainment on the planktonic community of North Arm, a comprehensive program to sample plankton in North Arm was recommended and developed during the referral process in 2018. The monthly phytoplankton, chlorophyll-a, zooplankton and fish eggs and larvae (ichthyoplankton) sampling program was initiated in December 2018, two months prior to the release of the Ministerial Guidelines for the EES.

This technical report provides the results of a 13-month study on ichthyoplankton in the project area. It is one of three reports on the plankton, the others being the phytoplankton (microscopic plants) and zooplankton (planktonic macroinvertebrates).

3 Sampling for EES

The ecosystem sampling studies for the EES were designed to optimise integration of the hydrodynamic modelling and particle entrainment modelling with a factual understanding of plankton community spatial and temporal variability, and dynamics in Lower North Arm of Western Port to assess the effects of entrainment on plankton populations from Berth 2 at Crib Point Jetty situated in the main channel in Lower North Arm, and implications for the Western Port ecosystem.

The key interest of the studies was to:

1. Characterise the key components of the North Arm Plankton community
2. Identify spatial patterns along or across the main channel of North Arm where the FSRU will be located that may indicate sensitivity of local populations to entrainment or cold-water discharge
3. Identify seasonal patterns that may inform assessment of impacts of the project under differing seasonal intake and discharge scenarios

The EES plankton studies recognised that water depth, water exchange, current speed, turbulence and proximity of seabed habitats are strong influences on the composition of plankton likely to pass the FSRU intake. The FSRU berthed at Crib Point Berth 2 would be located approximately 600 m offshore from the low tide mark, more than 500 m from the 2 m depth contour and approximately 450 m and 330 m offshore from the 5 m and 10 m depth contours (Chart Datum), respectively. The lower boundary of the nearest saltmarsh-mangrove community southeast and northeast of the Jetty would be more than 1,100 m from of the FSRU intake. The intake on an FSRU at Crib Point jetty would be located approximately 450 m offshore from the likely lower limit of subtidal seagrasses at Woolies Beach at Crib Point.

The studies aimed to document the planktonic character of the water mass of the main North Arm Channel that would be entrained by the FSRU. The methods used were those typically used to quantitatively sample phytoplankton, mesozooplankton (including some meroplankton) and ichthyoplankton (including larger invertebrate meroplankton). The equipment, methods and timing did not target particular species. All plankton sampling used depth integrated samples to sample the same depth band of the water column that would be drawn into the FSRU heat exchange intakes.

The plankton study design was based on the 1982 to 1984 zooplankton investigations of Western Port and Port Phillip Bay (Kimmerer and McKinnon 1985, 1987a, b, c), which provide spatial and temporal information on zooplankton in East Arm and the Western Entrance.

In the EES study, plankton sampling was designed to provide spatial and temporal information comparable between different trophic levels. Samples of phytoplankton, zooplankton and ichthyoplankton were collected monthly for a 13-month period (December 2018 to December 2019) along approximately 19 km of the Lower North Arm Channel.

Samples were collected at approximately monthly intervals using standard methods (see Section 3.3) at eight locations in spring and seven in summer, autumn and winter from December 2018 to December 2019. A total of four surveys were conducted in summer (n=4) and three surveys in spring, autumn and winter (n=3), to provide seasonal replicates at sampling sites. These seasonal replicates allowed for a seasonal analysis of the plankton community in the Lower North Arm.

3.1 Sites

The location of monthly sampling sites is shown Figure 1. Sites were distributed along a central north-south axis from the north of Lower North Arm southward to the Confluence Zone; and a west-east axis from the 10 m depth contours on the west of Lower North Arm at Crib Point eastward to the 10 m contour on the eastern side of the main channel.

The north-south axis sites were positioned to document spatial variability along the tidal gradient in Lower North Arm: waters in the south of Lower North Arm are exchanged with Bass Strait water on a shorter time-scale than those in the north of Lower North Arm. These sites documented the plankton community at sites from near the extensive intertidal and shallow subtidal seagrass beds in Upper North Arm, to sites near the wave and current exposed deep channels in the Confluence Zone.

Sites along the north-south axis were CPN2, CPN1, CPC1, CPS1 and CPS2. The location of some sites was adjusted during the program as understanding of the scale of spatial variability grew while ensuring each survey could be completed in a single day. Site CPN1 is near a long-term water quality monitoring site used by EPA Victoria (Hastings) while site CPC1 is in the middle of the channel directly east of Crib Point Jetty Berth 2.

Site CPS1 at the south end of lower north arm was expected to show Bass Strait influence, particularly at high tide, due to its proximity to the Confluence Zone and Western Entrance segments. The first six months of the sampling program showed conditions at CPS1 were quite similar to other lower north arm sites regardless of tide. Therefore, site CPS2 in the confluence zone was added from August 2019 to assess plankton with greater Bass Strait influence.

Sites on the east-west axis were designed to document spatial variability between waters over different benthic habitats across the main North Arm channel at Crib Point. Sites on the 10 m contour east (CPE10) and west (CPW10) of Crib Point are each around 100 m from shallow subtidal and intertidal seagrass beds. The Crib Point Berth 2 site is at the location of the proposed FSRU where entrainment will occur and is adjacent to the jetty habitat created by the jetty piles. CPC1 represents mid-channel habitat. Selection of the 10 m depth contour for the east and west sites also considered extent of discharge plumes from the FSRU predicted during preparation of the EES referral: the cooler-water FSRU discharge will form a seabed or mid-water plume at depths greater than 10 m.

As discussed below, the timing of surveys was not synchronised with tides, so that the water body sampled at any of the sites on any sampling occasion could have originated from up to 7 km kilometres over the six hours prior to sampling. This essentially randomised the spatial horizontal position of the samples and was consistent with the approach of Kimmerer and McKinnon (1985, 1987a). This allows characterisation of monthly variation in ichthyoplankton population in Lower North Arm with replication of monthly samples (n=7,8).

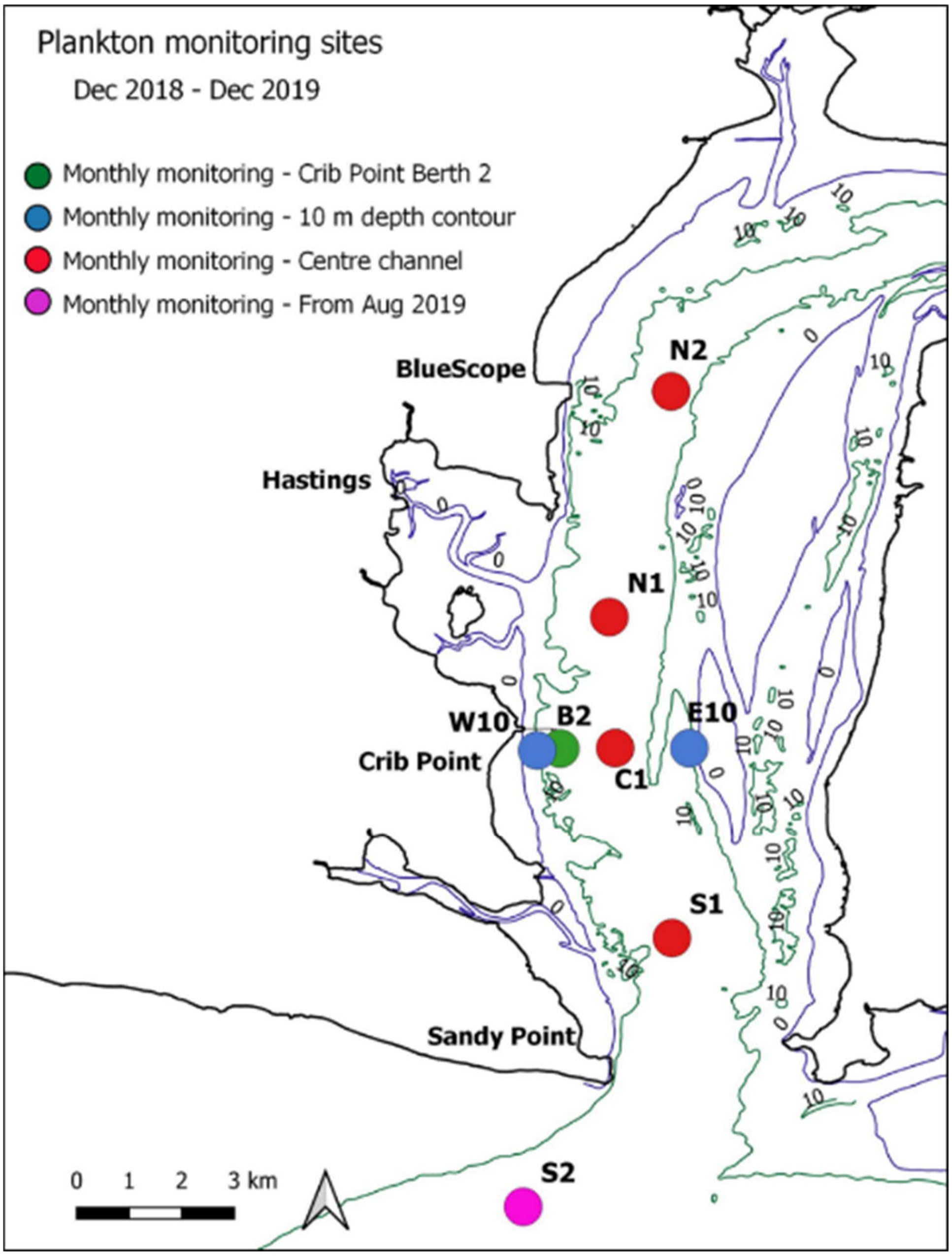

Figure 1. Plankton monthly sampling sites in Lower North Arm 2018-19, Western Port

3.2 Timing

Phytoplankton, zooplankton and ichthyoplankton were sampled at each monthly site visit. Plankton at all sampling sites were sampled at approximately monthly intervals from December 2018 and December 2019 inclusive (13 surveys).

Monthly samples were collected during daytime only, when weather was suitable within approximately one week the change in month. Mobilising to site, launching, sampling all sites, preserving samples and demobilising took a whole day per survey. Hence, surveys or sampling were not synchronised with tides in any way.

Individual samples (phytoplankton, zooplankton, ichthyoplankton) were collected during the same site visit. Phytoplankton and zooplankton samples were usually collected simultaneously. The vessel was usually relocated back to the approximate sample site position between the ichthyoplankton sample collection and the phytoplankton and zooplankton samples due to drift during sampling.

Each monthly survey was separated by a minimum of 3 and a maximum of 6 weeks. The sampling schedule is shown in Table 1.

Table 1. Monthly plankton sampling summary, December 2018 to December 2019

Monthly Survey	Date	S2	S1	B2	W10	E10	C1	N1	N2
Survey 1	11/12/2018		z,i,p	z,i,p	z,i	z,i	z,i,p	z,i,p	z,i,p
Survey 2	10/01/2019		z,i,p	z,i,p	z,i	z,i	z,i,p	z,i,p	z,i,p
Survey 3	4,11/02/2019		z,i,p	z,i,p	z,i	z,i	z,i,p	z,i,p	z,i,p
Survey 4	07/03/2019		z,i,p	z,i,p	z,i	z,i	z,i,p	z,i,p	z,i,p
Survey 5	04/04/2019		z,i,p	z,i,p	z,i	z,i	z,i,p	z,i,p	z,i,p
Survey 6	03/05/2019		z,i,p	z,i,p	z,i	z,i	z,i,p	z,i,p	z,i,p
Survey 7	07/06/2019		z,i,p	z,i,p	z,i	z,i	z,i,p	z,i,p	z,i,p
Survey 8	03/07/2019		z,i,p	z,i,p	z,i	z,i	z,i,p	z,i,p	z,i,p
Survey 9	02/08/2019	z,i,p	z,i,p	z,i,p	z,i	z,i	z,i,p	z,i,p	z,i,p
Survey 10	03/09/2019	z,i,p	z,i,p	z,i,p	z,i	z,i	z,i,p	z,i,p	z,i,p
Survey 11	1/10/2019	z,i,p	z,i,p	z,i,p	z,i	z,i	z,i,p	z,i,p	z,i,p
Survey 12	30/10/2019	z,i,p	z,i,p	z,i,p	z,i	z,i	z,i,p	z,i,p	z,i,p
Survey 13	11/12/2019	z,i,p	z,i,p	z,i,p	z,i	z,i	z,i,p	z,i,p	z,i,p
Total number of surveys		5	13	13	13	13	13	13	13

z = zooplankton, i = ichthyoplankton, p = phytoplankton

3.3 Ichthyoplankton sampling methods

The program aimed to optimise the collection of data that would inform the nature, seasonality and horizontal spatial patterns of fish eggs and larvae in relation to assessing the potential effects of entrainment on plankton communities in Lower North Arm of Western Port. Sampling methods for the 13-month, monthly ichthyoplankton sampling program were developed by CEE in consultation with Prof. Greg Jenkins, University of Melbourne. Professor Jenkins analysed the ichthyoplankton data and provided a report on the characteristics and ecology of the ichthyoplankton community in Lower North Arm of Western Port based on the 2018-19 sampling program. His report is appended to this report.

The methods also considered the practicalities of sampling in the spatial dimensions, water depths and strong tidal currents of Western Port, sorting effort and other information being collected (phytoplankton and zooplankton data, hydrodynamic modelling, water temperature logging) that would add to the understanding of patterns in the ichthyoplankton data

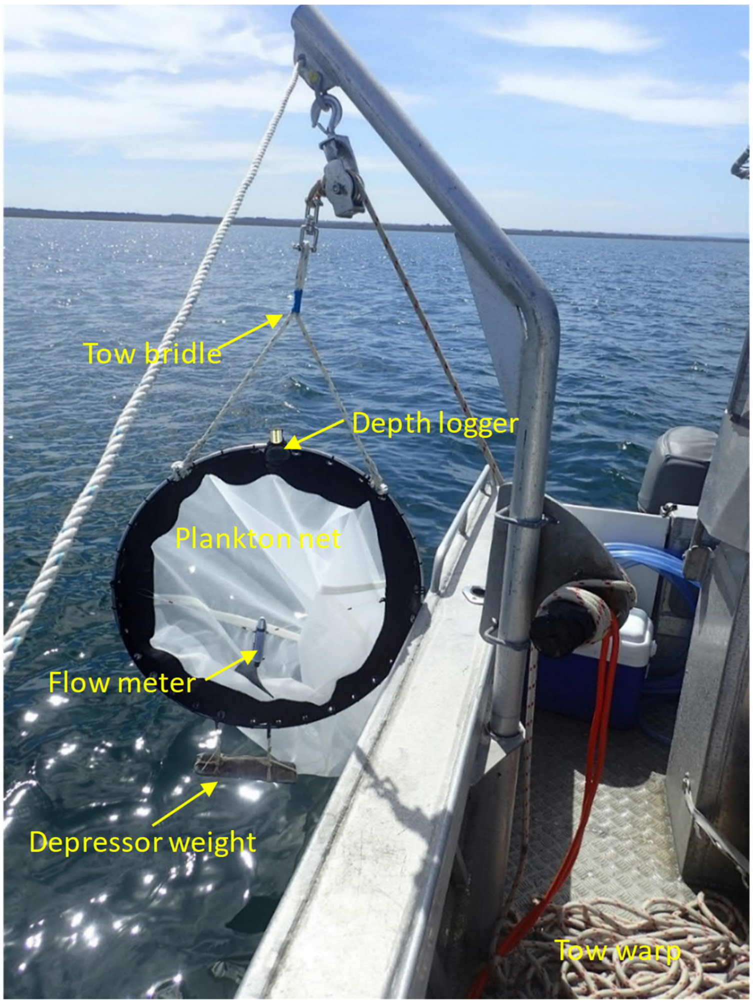

Figure 2. Ichthyoplankton sampling equipment

Ichthyoplankton were sampled using a conical net made from 500 µm mesh fixed to a rigid metal hoop. Sampling in the early parts of the program used a 1 m diameter, 5 m long net, while all subsequent sampling used a 0.8 m diameter, 4 m long net. A depth logger was attached to the top of the neck mouth to record sampling depth. A General Oceanics mechanical digital flow meter with a low velocity impeller was fixed across the mouth of the net to measure the flow of water passing through the net. A 1.5 kg depressor weight was attached to the bottom of the net mouth (Figure 2). A 100 m long warp (rope) marked at 4 m increments was attached to a bridle on the top of the net mouth for towing. The cod end was 100 mm diameter and 200 mm long. A 1.5 kg weight was attached to the cod-end to keep it level with the rest of the net during tows.

All ichthyoplankton samples were collected using a stepped-oblique tow from near the surface to near the seabed and back to the surface. This gave each tow a parabolic depth profile. During the first surveys, an inclinometer was used to measure the warp angle and adjust tow speed to the depth of each tow step, and maximum depths were checked from the depth logger following each tow to determine standard vessel in-water speeds in the tidal currents. The net was towed at a steady speed of 1 to 1.5 knots through the water (in-water average of 0.64 m/s) and each tow lasted 10-15 minutes.

The net was raised and lowered during the tow using the marked warp. The 4 m warp increments provided depth-steps of approximately 2 m. The tow period at each depth was adjusted according to total water depth at each site, so that the total volume sampled was around 350 m^3 for all tows, the exact volume for each tow being calculated from the net flow meter. Maximum depths were confirmed from the depth logger on completion of each tow. Depth profiles for all tows were logged.

Upon completion of each tow the sides of the net were rinsed from the outside from the top to the bottom of the net. Samples were concentrated by draining excess water through the side of the net and rinsing ichthyoplankton back into the cod-end.

One ichthyoplankton sample was collected at each site in each survey and fixed in 5% v/v buffered formalin. Clove oil was used to euthanise juvenile or post-larval fish, and Syngnathids where present, prior to fixing in formalin.

3.4 Laboratory processing of samples

Preserved ichthyoplankton samples were initially sorted by CEE scientists who separated and counted all fish eggs, fish larvae, pipe fish (Syngnathids) and cephalopods. For samples with very large numbers of eggs, the total number was estimated by removing all eggs to a gridded sorting tray, counting the fish eggs in 10 randomly distributed grid-cells and scaling the number up to the area of the tray. A Folsom splitter was tested for a number of samples but the macroalgae and seagrass leaves that fouled most samples prevented accurate splits.

The fish eggs, fish larvae, Syngnathids and cephalopods were sent to an ichthyoplankton specialist (Dr. A. Miskiewiz) at the Australian Museum for identification and archiving. Larvae were identified to the lowest practical level, which was typically genus or species. Species for which larval characters are undescribed were differentiated into families with lower classification based on morphological characteristics. In some cases, the species of specimens for which larvae are only described to family taxonomic level could be inferred based on the known distribution of species within than family.

Eggs of pilchards and anchovy were identified based on egg morphology but identification of the species for other fish eggs is not possible.

3.5 Data analysis

Flow metre data were used to calculate the volume (v) of sea water that had passed through the net for each sample using the formula:

$$v = a*n*c$$

where a = area of net mouth (m^2), n = flow meter number of rotations and c = rotor constant/999999.

From these data species counts were standardised to number per 1000 m^3.

As discussed above, the key interests of the studies were to:

1. Characterise the key components of the North Arm Plankton community
2. Identify spatial patterns along or across the main channel of North Arm where the FSRU will be located that may indicate sensitivity of local populations to entrainment or cold-water discharge
3. Identify seasonal patterns that may inform assessment of impacts of the project under differing seasonal intake and discharge scenarios.

3.5.1 Monthly community composition

The monthly monitoring program at the array of sample sites is shown in Table 1. Mean monthly abundance and standard error of the mean (mean± SEM) of phyto-, zoo-, and ichthyo- plankton taxa in Lower North Arm was estimated for thirteen months using the number of samples shown in Table 2. The table shows that the number of samples (n) for calculation of mean and standard error ranges from 5 for the first eight months of monitoring phytoplankton to 8 for the last five months of monitoring zooplankton and ichthyoplankton.

Table 2. Number of samples (n) used for monthly mean and SEM calculations Lower North Arm Dec 2018 to Dec 2019

Plankton	Dec 2018 to Jul 2019	Aug 2018 to Dec 2019
Phytoplankton	5	6
Zooplankton	7	8
Ichthyoplankton	7	8

3.5.2 Spatial patterns in Lower North Arm

Annual spatial pattern at monitoring sites

Spatial patterns between monitoring sites in Lower North Arm were demonstrated for key biota from annual mean and standard errors calculated from monthly data (Table 1). Table 3 shows that the number of samples (n) for calculation of mean annual abundance and standard error of the mean (mean± SEM) of phyto-, zoo-, and ichthyo- plankton taxa at monitoring sites in Lower North Arm ranged from 5 at site S2 to 13 at the major monitoring sites.

Table 3. Number of samples (n) used for annual mean and SEM calculations Lower North Arm monitoring sites Dec 2018 to Dec 2019

Plankton	S2	S1	B2	W10	E10	C1	N1	N2
Phytoplankton	5	13	13			13	13	13
Zooplankton	5	13	13	13	13	13	13	13
Ichthyoplankton	5	13	13	13	13	13	13	13

Seasonal spatial pattern at monitoring sites

Seasonal spatial patterns between monitoring sites in Lower North Arm were demonstrated for key biota from seasonal mean and standard errors calculated from monthly data (Table 1). The table shows that the number of samples (n) for calculation of mean annual abundance and standard error of the mean (mean± SEM) of phyto-, zoo-, and ichthyo- plankton taxa at monitoring sites Lower North Arm was n=3 for autumn, winter and spring and n=4 for summer.

3.5.3 Further data presentation and use

Environment Effects Statement

Further discussion of the results in the context of hydrodynamic, entrainment and discharge modelling, zooplankton ecology, environmental guideline values and impact assessment are provided in separate EES related documents. The assessment of impacts on marine ecosystem components including plankton communities, is a risk-based, 'likelihood X consequence' process informed by statistically determined environmental guidance values (for temperature, chlorine concentration and entrainment proportion with respect to natural flushing), hydrodynamic, entrainment and exposure modelling and understanding of ecological characteristics documented in the technical studies.

Baseline documentation and impact assessment monitoring

The environmental data collected during EES studies, such as those presented in this report, will inform selection of environmental indicators, and the design and statistical power of baseline and operational compliance monitoring programs if the project proceeds.

3.6 QAQC

Quality assurance processes samples collected during this task included:

- Sampling equipment was rinsed with fresh seawater prior to sampling, and sampling equipment was rinsed with fresh water following each survey to prevent cross-contamination of samples
- Only new sample containers were used
- The time, date, GPS position and sampling depth was recorded for each sample, along with details of tide state, weather and sea-state
- Samples were delivered to the analysing laboratory along with chain of custody documentation detailing the sampling dates, sites and sampling depths for each sample.
- Field data were compiled into a database within a few days of sampling
- Laboratory procedures were carried out by academic specialists

Quality control for data collected during this task included:

- Checks that the field recorded GPS positions for each sample matched the planned (regular) site location
- Data returned by the laboratory were cross checked against field records and compiled into a database
    - o Checks to ensure that the same number of data provided by the laboratory were entered into the database
    - o Checks for consistency of data coding (site and sample identifications, taxonomic nomenclature, units)

4 Results

Table 4 summarises results of the sampling program. The table lists average abundances (concentration per 1000 m^3) for ichthyoplankton (fish larvae and post-larvae), fish eggs and cephalopod paralarvae in each survey. The table also includes average abundance of fish larvae in the ten most numerous families.

Table 4. Summary of monthly average ichthyoplankton abundance

	Dec-18	Jan-19	Feb-19	Mar-19	Apr-19	May-19	Jun-19	Jul-19	Aug-19	Sep-19	Oct-19	Nov-19	Dec-19	13 Month Total	
n (samples)	7	8	8	8	8	8	8	8	8	9	8	8	9	105	
Ichthyoplankton	574	183	62	19	9	3	4	2	4	62	101	225	473	1721	
Fish Eggs	120	30	3	16	2	3	7	28	214	204	146	161	115	1047	
Cephalopods	70	58	39	11	11	3	0	0	0	0	2	43	47	284	
Total Families	18	12	11	9	5	5	2	6	5	8	14	15	15	57	
Top-ten Ichthyoplankton Families (number of species)														Total	%
Gobiidae (14)	513.7	154.7	34.6	6.3	0.1		0.8	0.4	0.8	50.8	27.0	164.6	435.0	1389	81
Syngnathidae (8)	23.4	19.4	15.6	7.8	7.8	2.3	3.0	1.1	1.8	1.6	0.9	5.3	7.7	97	6
Tetrarogidae (1)	0.2								1.3	7.9	53.6			63	4
Tripterygiidae (1)	0.3									1.2	11.0	19.3	6.1	38	2
Gobiesocidae (6)	3.0	0.3	0.3	0.6		0.1			0.1	0.3	3.3	17.6	4.2	30	2
Aracanidae (1)	19.1	3.9	0.4	0.1									2.8	26	1.5
Monacanthidae (5)	2.3	2.1	6.4	1.9	0.8						1.0	1.8	7.6	24	1.4
Apogonidae (6)	4.0	0.1	2.6	0.5	0.1								1.1	8	0.5
Blenniidae (2)	0.6	0.4	0.6	0.1							0.1	3.3	2.1	7	0.4
Scorpaenidae (2)												6.6	0.4	7	0.4

The abundance of fish larvae, fish eggs and cephalopods have shown large seasonal variations, with maximum numbers during summer and minimum numbers over winter.

Up to 21 fish larvae species were identified at a single survey. In contrast, to the diversity of families represented by larval fish, cephalopod larvae were dominated by one species, the pygmy squid, *Xipholeptos notoides*, together with a few individuals of the southern dumpling squid, *Euprymna tasmanica*.

Fish larvae came from 29 families, dominated by the Gobiidae (gobies) that comprised 81 per cent of the ichthyoplankton collected. Gobiidae has also been the most species rich group, with 14 species identified. Gobiidae were particularly abundant in the samples collected at W10 m in December 2018 and at N1 site in December 2019, which had a large influence on the average for the December samples.

Syngnathiidae (pipefish, seahorses) were the second most abundant ichthyoplankton family representing 6 per cent of the ichthyoplankton. They were the second most diverse with 8 species identified. Note that many Syngnathids collected from the plankton were juvenile or adult fish, rather than larvae. Syngnathid abundance was highest in the December 2018 survey and has steadily decreased until October 2019 to only slightly increase in November and December 2019.

Tetrarogidae (fortesques or cobblers) accounted for 4 per cent of the ichthyoplankton collected but were represented by one species, the cobbler *Gymnapistes marmoratus.* Tetrarogidae were only observed in December 2018, and between July and October 2019.

The Tripterygiidae (triplefins) and the Gobiesocidae (clingfish) both accounted for 2 per cent of the ichthyoplankton. Only 1 species of Tripterygiidae was observed throughout the program period, while 6 clilngfish (Gobiesocidae) species were identified. Both families had their highest abundances recorded during Spring.

Aracanidae accounted for 1.5 per cent of the ichthyoplankton and were also represented by just one species, likely Shaw's Cowfish *Aracana aurita* which is common in Western Port. Aracanidae were only observed in the months of December (2018 and 2019) and January 2019, being most abundant in December 2018.

Monacanthidae (leatherjackets) accounted for 1.4 per cent of the ichthyoplankton and were represented by 5 species being most abundant during Summer and absent during Winter.

The remaining families all accounted for less than 1 per cent of the ichthyoplankton. Although in low abundance Apogonidae (cardinalfishes) was represented by 6 different species. All other families were represented by a single species with exception of the Blenniidae and Scorpaenidae (2 species each).

In total, 60 species of ichthyoplankton were identified, and 46 of those (77 per cent) have been from the ten most abundant families. The remaining species have been identified in single numbers from just one or a few samples.

Except for Monacanthidae (leatherjackets) and perhaps Tetrarogidae (cobbler), none of the top-ten families includes species that are targeted by recreational or commercial fishing.

Syngnathidae are listed on the Fisheries Act and EPBC Act to control harvesting for the aquarium and Chinese medicine industries. Engraulidae (anchovy) are an important food species for the little penguin *Eudyptula minor*.
Several species were identified that are targeted by recreational anglers or listed on the FFG Act or EPBC Act. All were present in low numbers and are listed separately in Table 5 along

with the total number of specimens identified throughout the sampling period. Leather jackets (*Meuschenia* sp.) were the most common fish that may be targeted by recreational anglers, followed by weed whiting (most likely *Haletta semifasciata,* blue weed whiting). Small numbers of flathead, flounder, cod, barracouta and garfish were collected, and one King George Whiting.

A larva from the family Retropinnidae was identified in September 2019 and is likely to be a larval Australian Grayling (*Prototroctes maraena*). The occurrence of this individual is discussed further in CEE's main EES Technical Report 2020.

Table 5. Species of recreational angling or conservation significance

Significance	Species	Common Name	2018-19 total
Recreational	*Meuschenia* sp.	Leather Jacket	42
Recreational	?*Halleta semifasciata*	Blue weed whiting	19
Recreational	Rhombosoleidae/Bothidae	Flounders	10
Recreational	Flathead	Flathead	9
Recreational	*Thyrsites atun*	Barracouta	4
Recreational	Hemiramphidae	Garfish	3
Recreational	Labridae	Wrasse	2
Recreational	Moridae	Cod	1
Recreational	*Genyptrus* sp.	Rockling	1
Recreational	*Sillaginoides punctatus*	King George Whiting	1
FFG/EPBC	?*Prototroctes maraena*	Australian Grayling	1

? denotes the 'most likely' species given the known distribution of species within the family.

4.1 Temporal patterns

The plots that follow illustrate the temporal patterns in ichthyoplankton, fish egg and cephalopod numbers during the program. The plots also show records for average monthly temperature (from monthly temperature measurements at each site) and monthly average daylength.

Figure 3 shows average fish egg concentrations in each month since December 2018. There were 441 fish eggs per 1000 m^3 in December 2018, declining to low numbers in February 2019 until June 2019. Numbers began to increase in July 2019, peaked in August 2019 and steadily decrease until the end of the sampling program. The large error bars indicate spatial variability, or patchiness, in egg concentrations. The increase in fish egg numbers coincided with increasing daylength following the winter solstice. Temperatures did not begin to increase until September, a month after peak egg concentrations occurred.

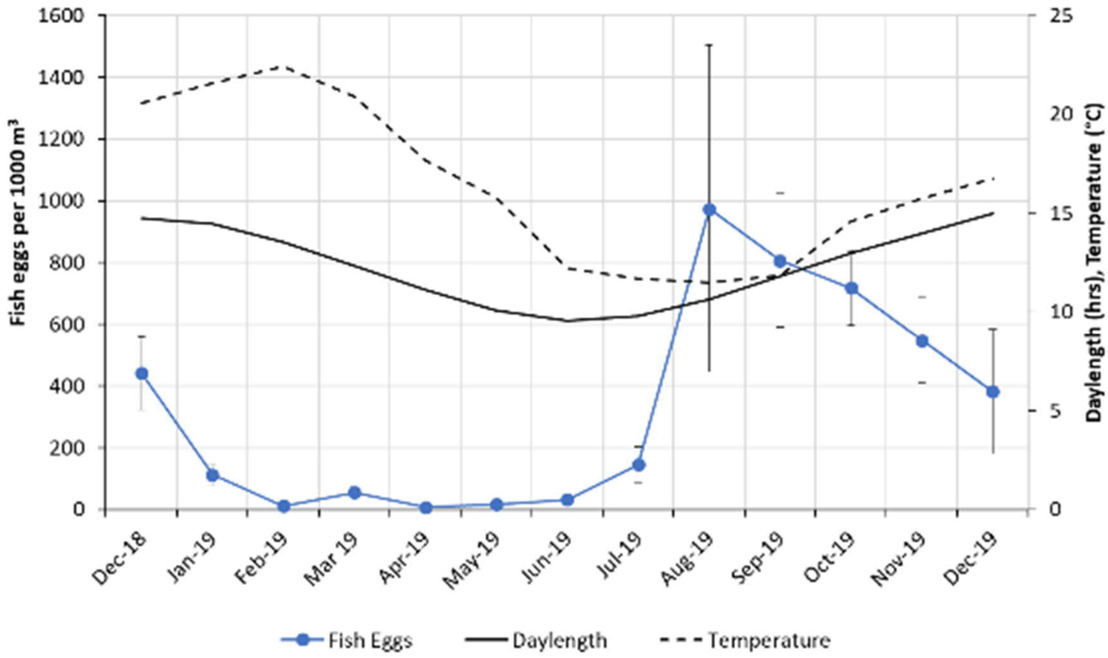

Figure 3. Average fish egg concentrations in lower north arm.
(mean ± se)

Figure 4 shows average fish larvae concentrations in each month since December 2018. December months had both the highest and most variable abundances with an average of 2225 fish larvae per 1000 m^3. Numbers declined steadily from January reaching its lowest in July (12 per 1000m^3). Fish larvae abundance remained low during winter and the spring increase occurred around one month after the August peak in fish egg concentrations.

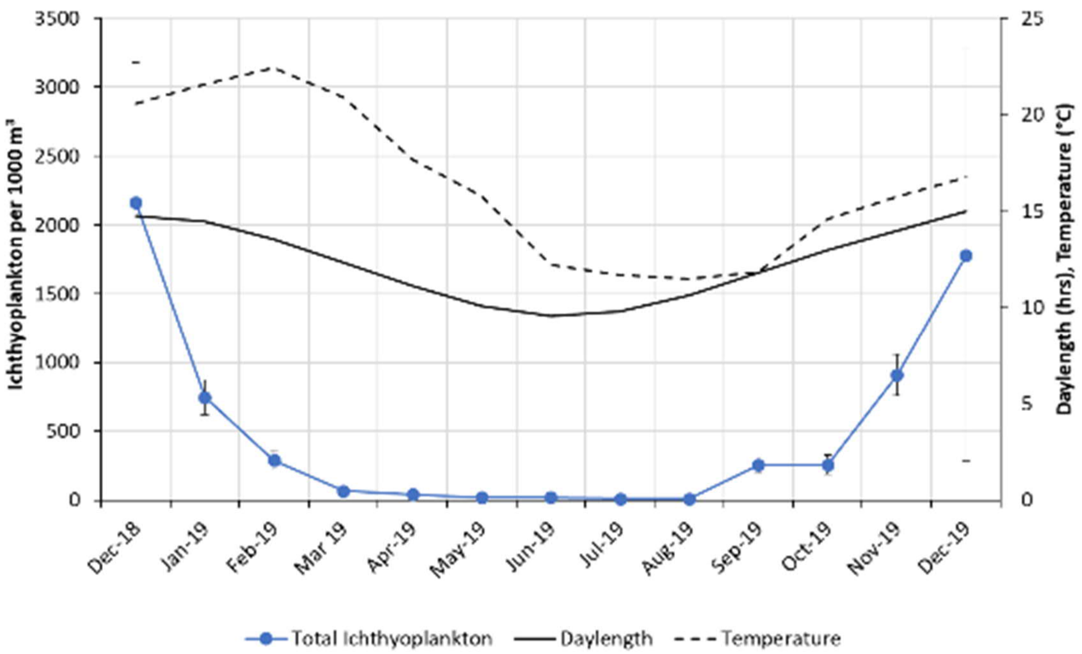

Figure 4. Average fish larvae concentrations in lower north arm
(mean ± se)

Fish larvae numbers do not relate strongly to either daylength or temperature: maxima, minima and inflexions in the seasonal pattern do not match either daylength or temperature curves.

Figure 5 shows the average cephalopod paralarvae concentrations in each month since December 2018. Most Cephalopod were *Xipholeptos notoides* (pygmy squid) paralarvae. This species of squid reaches a maximum size of around 25 mm mantle length and is strongly associated with seagrass. *X. notoides* attaches to seagrass using a specially adapted pad on its ventral side. *X. notoides* collected during this program may have been attached to drifting seagrass leaves commonly encountered in lower north arm.

Three cephalopod paralarvae specimens were *Euprymna tasmanica* (southern dumpling squid) and all came from the sample collected from site N2 in February 2019.

Neither Cephalopod are of fisheries significance, and neither is listed on the FFG Act or EPBC Act. Given its large numbers, *X. notoides* is likely an important predator of small invertebrates and fish larvae, and an important prey species for larger fish and invertebrates.

Cephalopod numbers were highest in December 2018 (259 per 1000 m^3), remained abundant through January and February, decreased to low numbers through Autumn before decreasing to negligible abundances from June to September 2019. Numbers began to increase in October 2019.

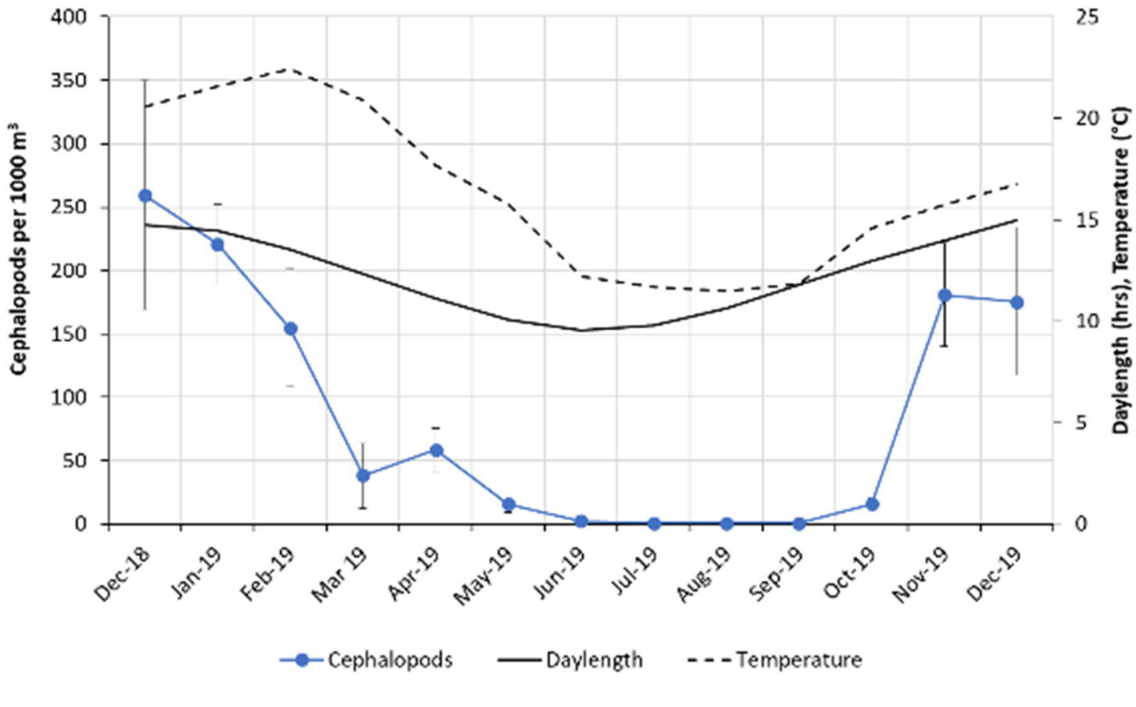

Figure 5. Average cephalopod concentrations in lower north arm
(mean ± se)

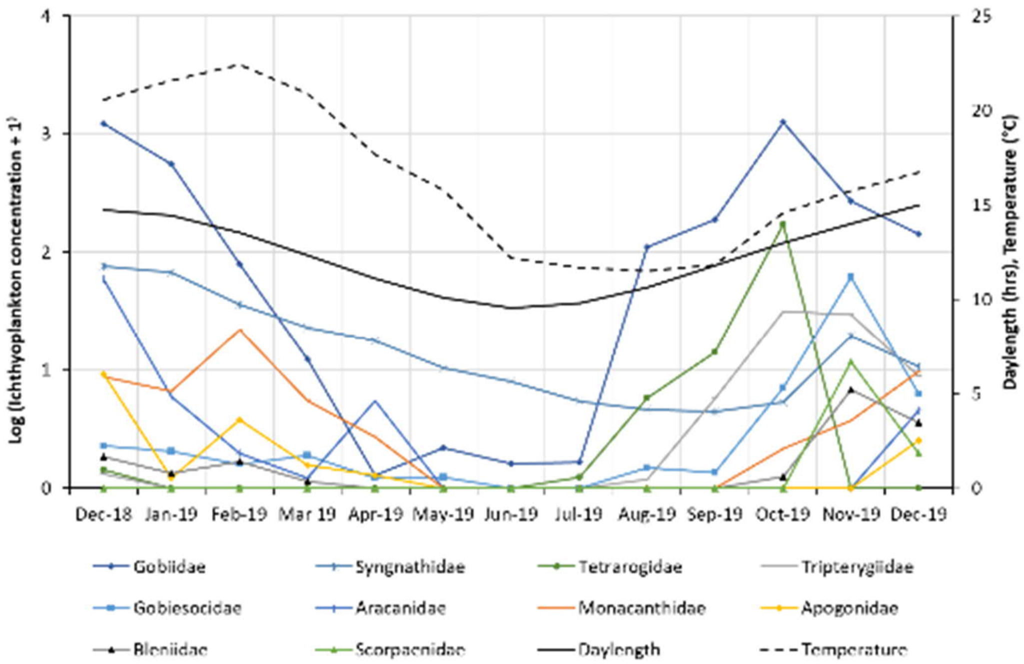

Figure 6. Average abundance of top ten ichthyoplankton families
(SE bars omitted for clarity of data presentation)

Figure 6 shows the average monthly concentration of the ten most abundant ichthyoplankton families from December 2018 to December 2019. Note the log (n+1) scale. The chart shows that the ichthyoplankton community was dominated by Gobiidae throughout summer and were 10 times more abundant than any other family in December 2018. Goby abundance decreased to low levels over winter but increased more than 10-fold between August and September 2019 to again dominate the ichthyoplankton community. Patterns in Gobiidae abundance follow patterns in daylength.

Syngnathidae abundance decreased steadily from December 2018 to October 2019 to increase again in early summer. Syngnathidae were the dominant family through autumn and winter. This may be due to the presence of juveniles or small adults associated with drifting seagrass over this period.

Although presenting negligible concentrations earlier in the program, Tetrarogidae abundance increased rapidly from July 2019 to be the second most abundant family until October.

Tripterygiidae were rarely found until August 2019 but increased to become the third most abundant family during spring.

Gobiesocidae have had low abundance with less month to month variation than other taxa, though its abundance increased during spring and was the second most abundant family by November. The Aracanidae (boxfish) and Apogonidae (cardinalfish) had peak abundances in early summer (December 2018 and December 2019). Monacanthidae (leatherjackets) had peak abundance in late summer (February). Blenniidae and Scorpaenidae were comparatively present in low concentrations. Both families were not observed in autumn and winter samples and reached their maximum abundance in late spring (November).

Figure 7 shows the number of ichthyoplankton species in each month at each site. The number of species was highest in December 2019 (12-21 per sample), lowest from May to

July 2019 (1-3 per sample) and increased to 6-19 per sample during spring (September to October). The decrease and increase in species number closely matches patterns in daylength.

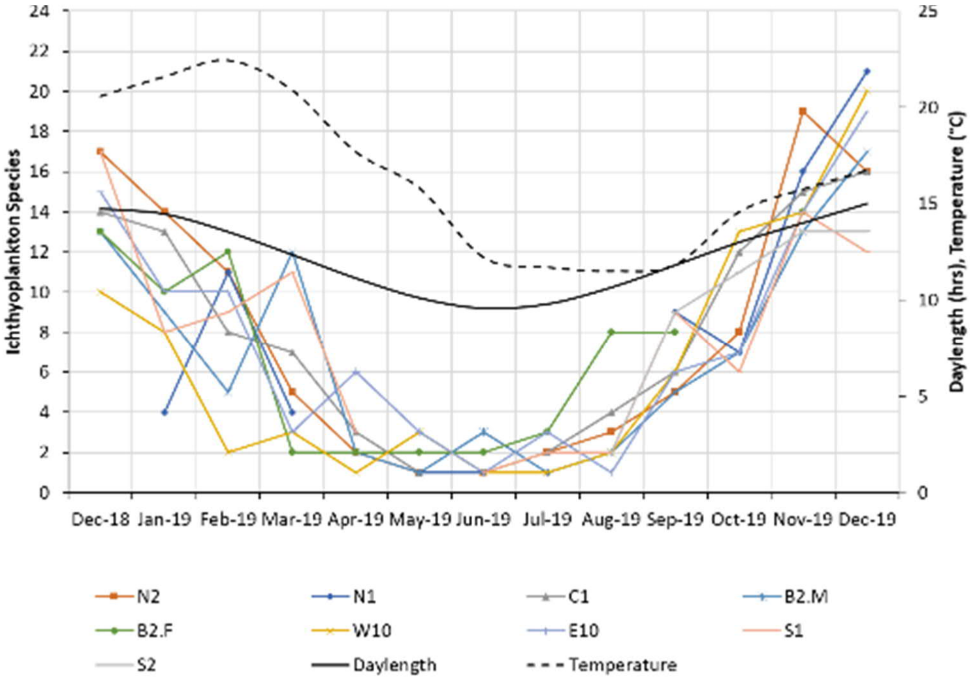

Figure 7. Number of ichthyoplankton species per sample
(SE bars omitted for clarity of data presentation)

Figure 8 shows seasonal patterns in ichthyoplankton (excluding fish eggs and cephalopods) concentrations per site. Abundances were highest in summer. During the December 2019 survey a remarkable high concentration of fish larvae was observed at site N1 (11816 larvae/1000m^3). Relative high concentrations were also observed during spring. Fish larvae concentrations show a patchy spatial distribution with no clear pattern.

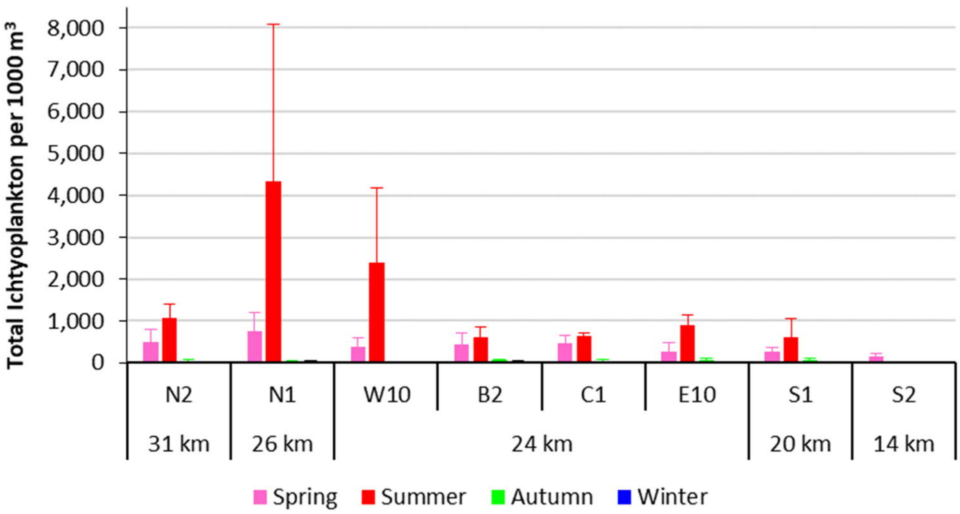

Figure 8. Seasonal average concentration of total ichthyoplankton per site
(Dec 2018 – Dec 2019, mean ± se)

Figure 9 shows spatial and temporal patterns in fish egg concentrations. Fish egg concentrations were highest in winter and spring. As with fish larvae, there were no

consistent spatial patterns or gradients in fish egg numbers, instead they show patchy distributions.

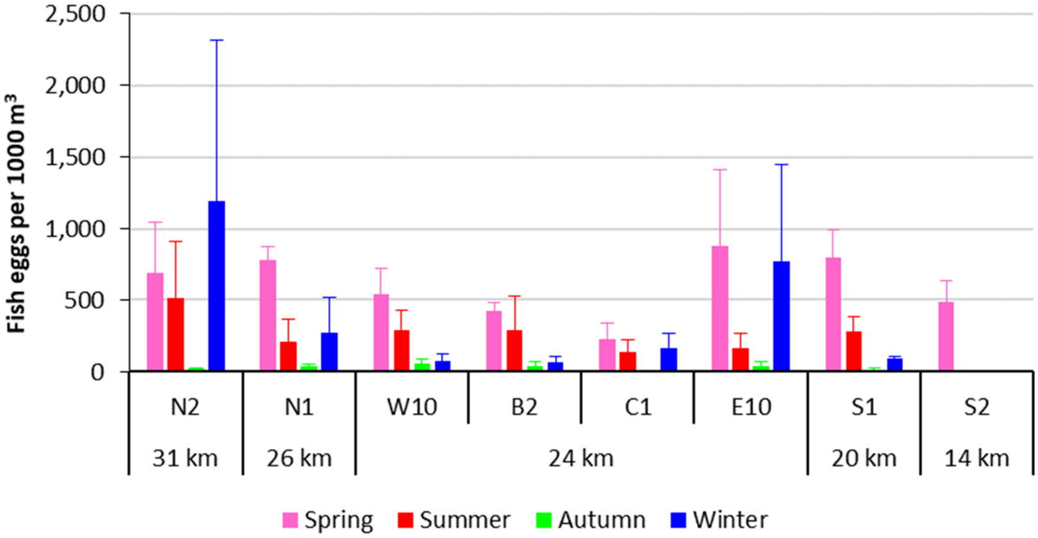

Figure 9. Seasonal average concentration of fish eggs per site
(Dec 2018 – Dec 2019, mean ± se)

The concentration of cephalopod larvae (Figure 10) was notably higher during Summer with no apparent spatial pattern. Relative high abundances were observed during Spring but unlike other fish larvae and fish eggs, cephalopod larvae were also found during Autumn surveys in concentration comparable to Spring.

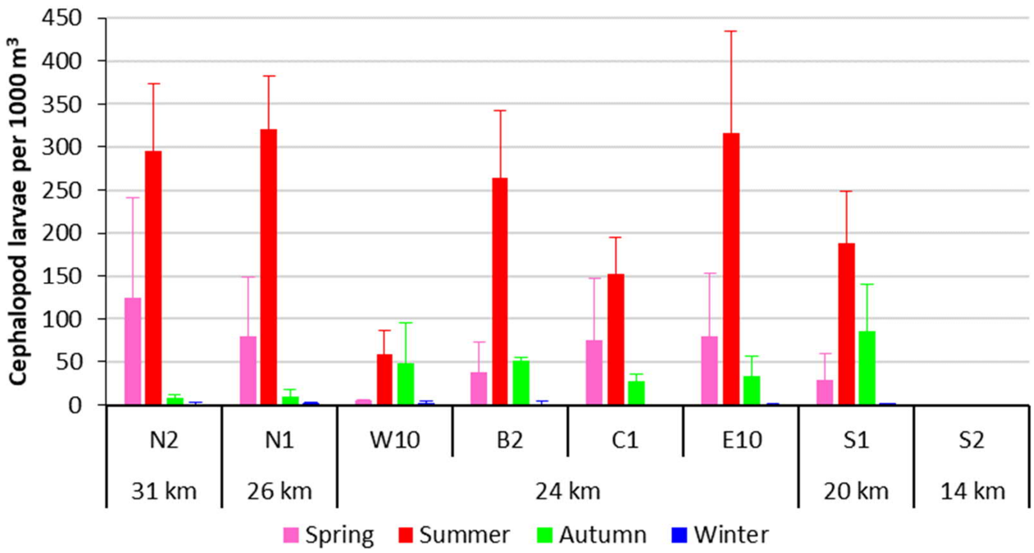

Figure 10. Seasonal average concentration of cephalopod larvae per site
(Dec 2018 – Dec 2019, mean ± se)

The average number of fish families represented as larvae was relatively even between sites but there was a strong seasonal pattern with more families recorded in summer and spring (Figure 11).

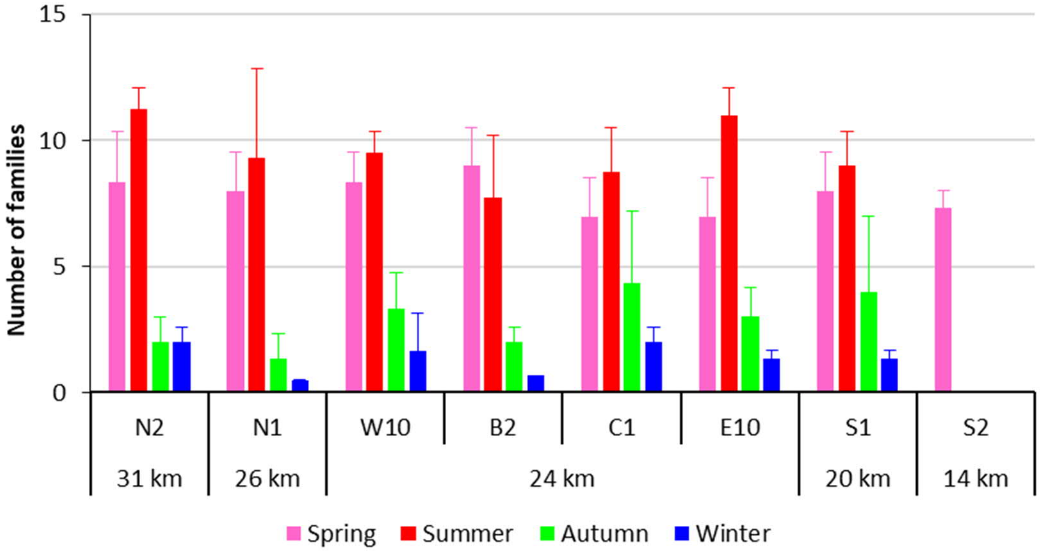

Figure 11. Seasonal average number of top ten families found at each site
(Dec 2018 – Dec 2019, mean ± se)

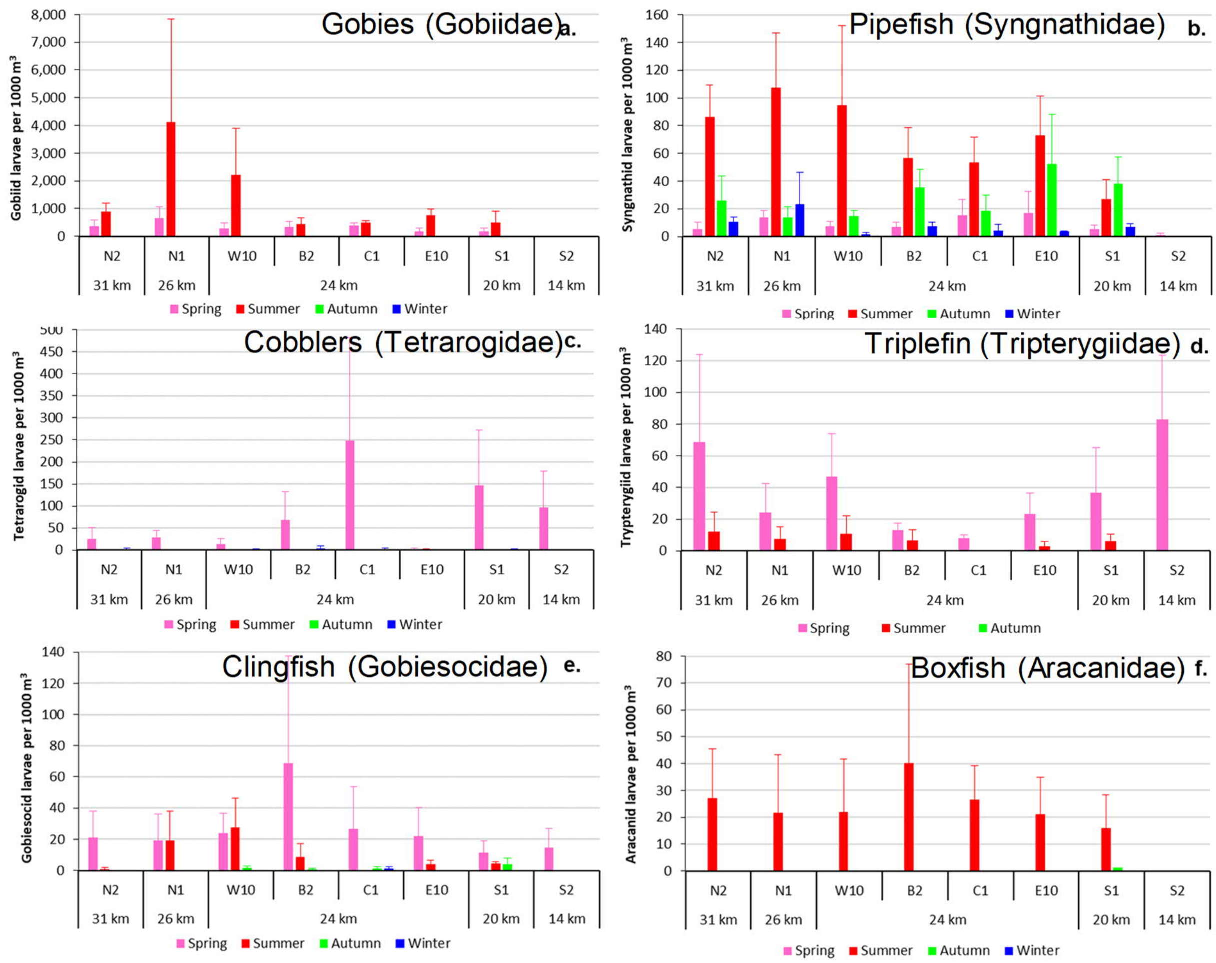

Figure 12. Seasonal average concentration of individual top ten families
(Dec 2018 - Dec 2019, mean ± se)

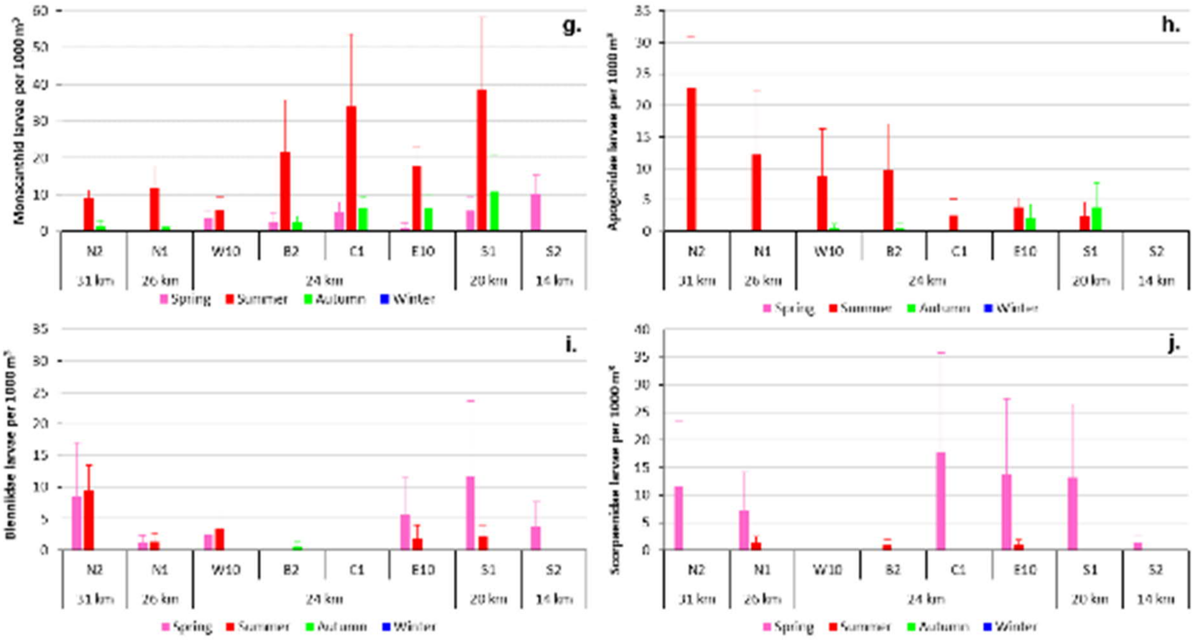

Fig 12. (*cont.*) Seasonal average concentration of individual top ten families

g. leatherjackets (Monacanthidae); **h.** cardinalfish (Apogonidae); **i**. blennies (Blenniidae); **j**. scorpionfish (Scorpaenidae)

The highest abundances of fish larvae for all top ten families were recorded during either summer or spring (Figure 12):

- The dominant gobiid larvae numbers were higher during summer and spring with south sites and E10 presenting the lower abundances (Figure 12a). Extremely high numbers of gobies were recorded as Sites W10 in December 2018 (7191 larvae/1000m^3) and at N1 in December 2019 (11526 larvae/1000m^3).
- The spatial distribution of pipefish (Syngnathidae) larvae was relatively even across the sites within each season (Figure 12b). Highest concentrations were recorded in summer with a tendency towards higher abundances at N2, N1 and W10 and lower abundances at S1 and S2. On the west-east transect, Syngnathid larvae were slightly more abundant at W10 and E10 than B2 and C1.
- Cobbler (Tetrarogidae) larvae showed strong spatial variability, with most larvae coming from sites C1 and the southern sites (Figure 12c).
- Triplefin (Tripterygiidae) larvae also showed strong spatial variability, with most larvae collected from the southern sites (Figure 12d).
- Clingfish (Gobiesocidae) larvae were mainly collected in spring, with highest concentration found at Crib Point (B2) (Figure 12e).
- Boxfish (Aracanidae) larvae were only recorded in summer samples and at a single site in March (S1) and presented a similar spatial pattern to Gobiesocid larvae (Figure 12f). They were relatively evenly distributed across sites, with exception of their absence at S2, and the highest concentrations were found at Crib Point site (B2).

Leatherjackets (Monacanthid) larvae tended to show higher abundances in summer towards the south, with highest abundance at S1 (Figure 12g). Spatial variability was also apparent for cardinalfish (Apogonidae) larvae that were mainly collected at the northern sites during summer (Figure 12h). Blennie (Figure 12i) and scorpionfish (Figure 12j) larvae were strong seasonal in their presence (spring and summer), but variable in their distribution.

4.2 General Patterns

Figure 3 and Figure 4 show the monthly abundance of fish eggs and larvae in Lower North Arm over period of the sampling program. Both show strongly seasonal patterns but demonstrate the sequence of eggs before larvae. The abundance of fish larvae in the Lower North Arm was strongly seasonal, with highest abundances in summer and spring and low abundance in autumn and winter. This pattern was largely a reflection of the seasonal pattern of abundance of the two dominant larval fish families, the gobies and the syngnathids, as well as numerous less abundant families.

The seasonal abundance of fish eggs showed a different pattern, with highest abundances in late winter – early spring. This difference between seasonal patterns of abundance for larvae and eggs is a reflection of the life histories of the dominant families.

The gobies lay benthic eggs and larvae hatch and enter the water column, while in the syngnathids the males brood the eggs on their bodies before larvae are released into the water column. The most abundant family that has the most common reproductive pattern for marine fish (spawning of pelagic eggs into the water column that hatch into pelagic larvae) was the Tetrarogidae, represented by a single species, the cobbler *Gymnapistes marmoratus*. Larvae of this species were most abundant in October and it is likely that the peak in egg abundance in August and September was largely a reflection of the spawning of this species.

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6 Appendices.

6.1 Table of monthly ichthyoplankton abundance

Table 6. Summary of average ichthyoplankton abundance per month

	Dec-18	Jan-19	Feb-19	Mar-19	Apr-19	May-19	Jun-19	Jul-19	Aug-19	Sep-19	Oct-19	Nov-19	Dec-19	13 Month Total	
n (samples)	7	8	8	8	8	8	8	8	8	9	8	8	9	105	
Ichthyoplankton	574	183	62	19	9	3	4	2	4	62	101	225	473	1721	
Fish Eggs	120	30	3	16	2	3	7	28	214	204	146	161	115	332	
Cephalopods	70	58	39	11	11	3	0	0	0	0	2	43	47	234	
Total Families	18	12	11	9	5	5	2	6	5	8	14	15	15	57	
														Total	%
Gobiidae (14)	513.7	154.7	34.6	6.3	0.1		0.8	0.4	0.8	50.8	27.0	164.6	435.0	1389	81
Syngnathidae (8)	23.4	19.4	15.6	7.8	7.8	2.3	3.0	1.1	1.8	1.6	0.9	5.3	7.7	97	6
Tetrarogidae (1)	0.2								1.3	7.9	53.6			63	4
Tripterygiidae (1)	0.3									1.2	11.0	19.3	6.1	38	2
Gobiesocidae (6)	3.0	0.3	0.3	0.6		0.1			0.1	0.3	3.3	17.6	4.2	30	2
Aracanidae (1)	19.1	3.9	0.4	0.1									2.8	26	1.5
Monacanthidae (5)	2.3	2.1	6.4	1.9	0.8						1.0	1.8	7.6	24	1.4
Apogonidae (6)	4.0	0.1	2.6	0.5	0.1								1.1	8	0.5
Blenniidae (2)	0.6	0.4	0.6	0.1							0.1	3.3	2.1	7	0.4
Scorpaenidae (2)												6.6	0.4	7	0.4
Tetraodontidae (2)	1.9	1.8		1.8	0.3			0.1				0.1	0.7	7	0.4
Odacidae (1)	2.4	0.1									0.1	2.9	1.0	6	0.4
Clupeidae (1)	0.1	0.3	0.1								1.5	0.8	1.8	5	0.3
Engraulidae (1)			1.0			0.5				0.1		0.1	1.8	4	0.2
Platycephalidae (1)	0.1	0.1				0.1					0.9	1.5	0.3	3	0.2
Sphyraenidae (1)	0.9											0.3	0.4	2	0.09
Rhombosoleidae (1)						0.1		0.1		0.2	0.8			1	0.07
Clinidae (1)	0.9													1	0.05
Pomacentridae (1)	0.7													1	0.04
Atherinidae (1)	0.3											0.4		1	0.04
Gempylidae (1)								0.3		0.1				0	0.02
yolk sac larvae (1)	0.1	0.1										0.1		0	0.02
Hemiramphidae (1)			0.3											0	0.02
Callionymidae (1)			0.1	0.1										0	0.01
Labridae (1)											0.3			0	0.01
Moridae (1)								0.1						0	0.01
Ophidiidae (1)									0.1					0	0.01
Silligatinidae (1)											0.1			0	0.01
Bothidae (1)											0.1			0	0.01

6.2 Monthly plots of less abundant species

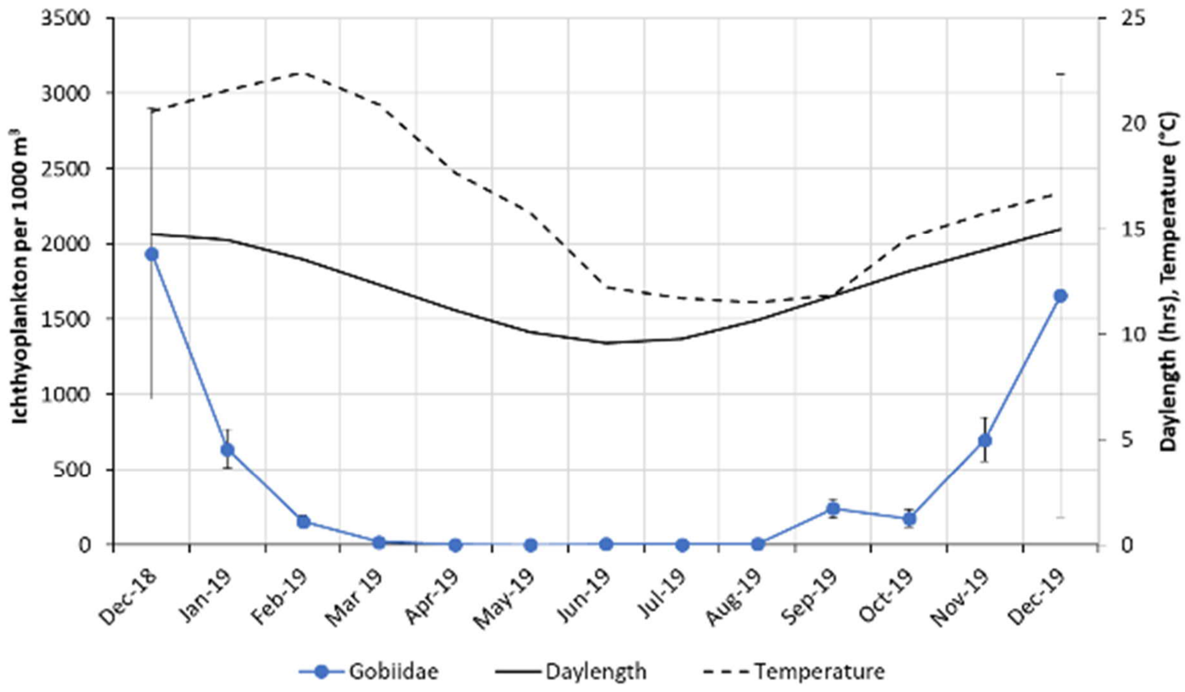

Figure 13. Goby (Gobiidae) abundance over time

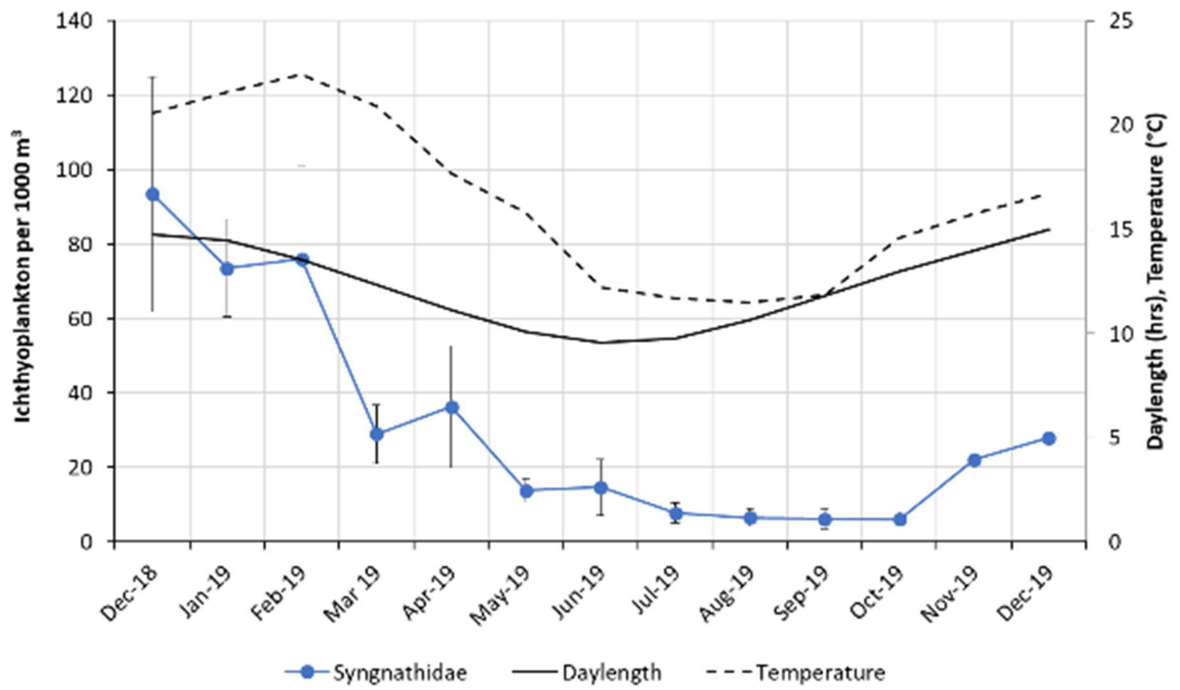

Figure 14. Pipefish (Syngnathidae) abundance over time

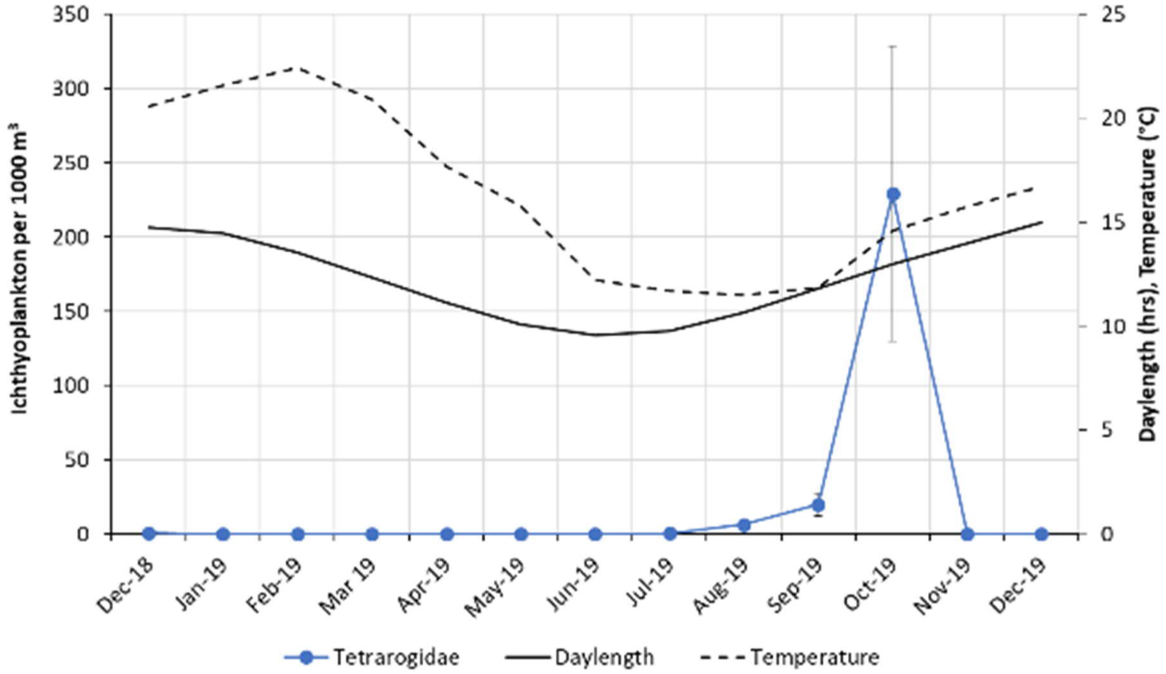

Figure 15. Cobbler (Tetrarogidae) abundance over time

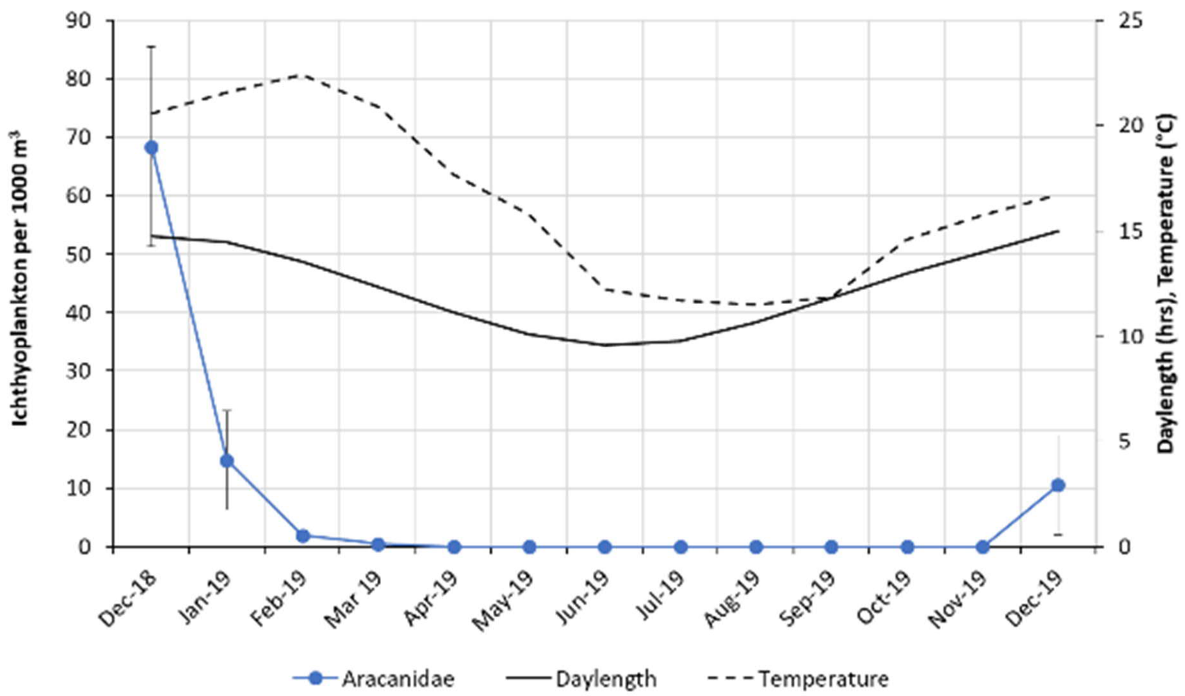

Figure 16. Boxfish (Aracanidae) abundance over time

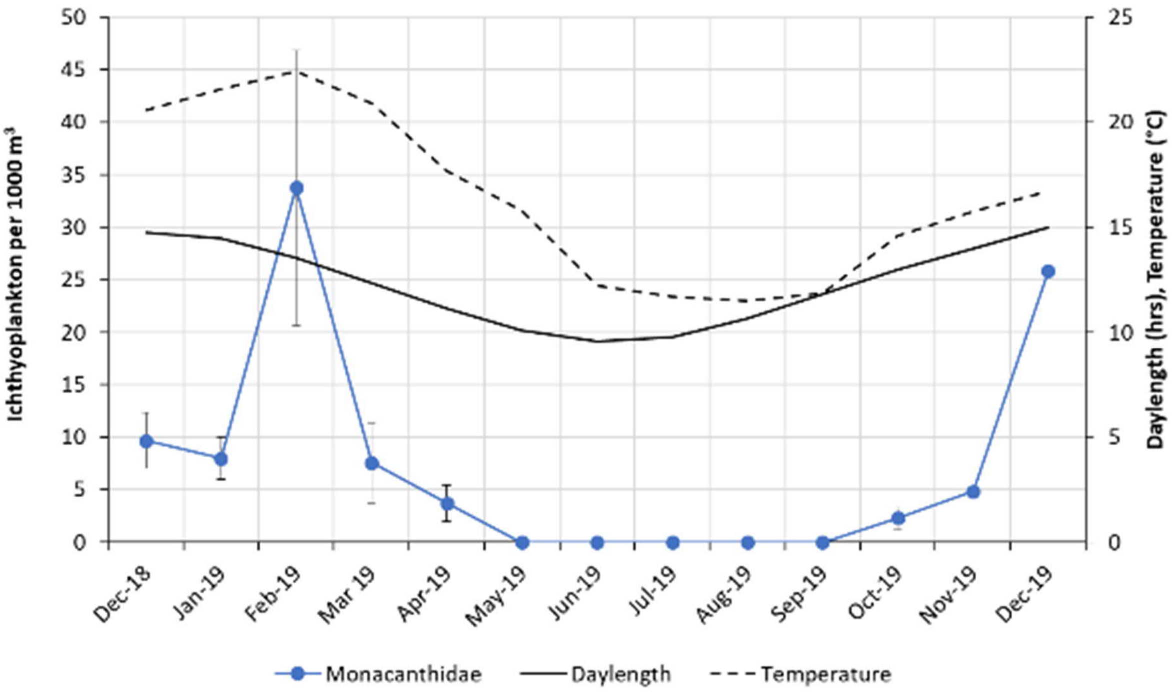

Figure 17. Leatherjacket (Monacanthidae) abundance over time

6.3 Seasonal plots toadfish and anchovies

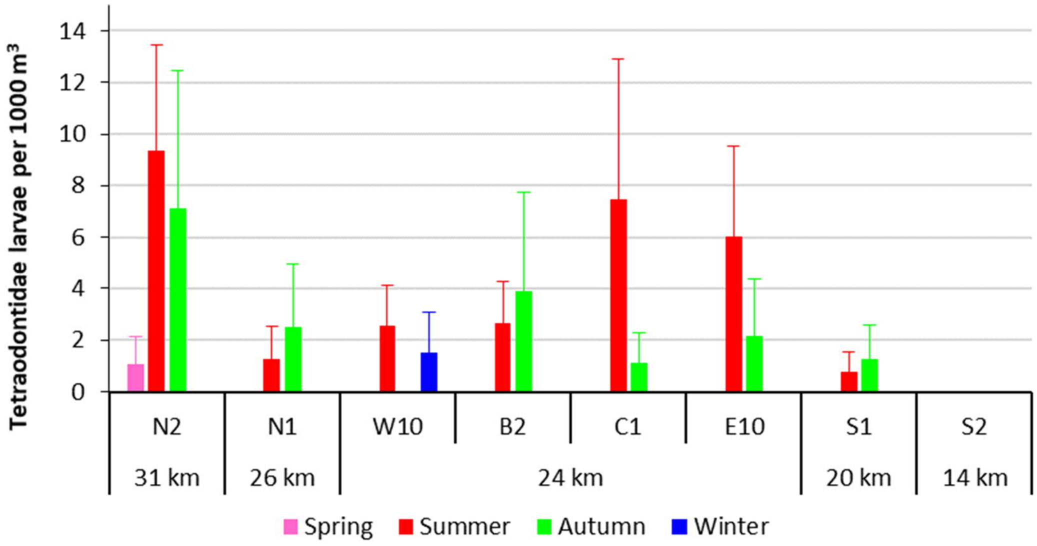

Figure 18. Seasonal average abundance of toadfish per site (Dec 2018 - Dec 2019)

(mean ± se)

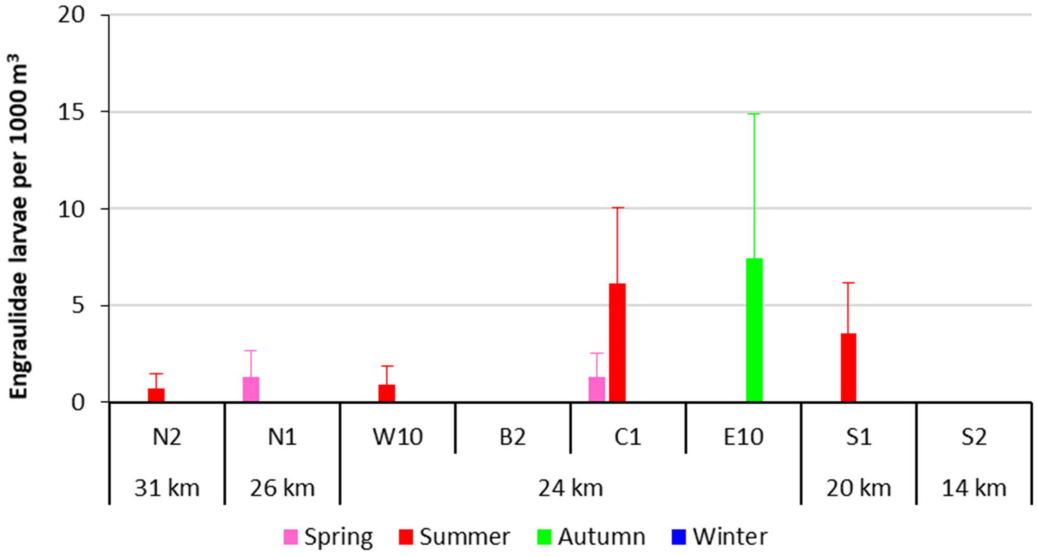

Figure 19. Seasonal average abundance of anchovy per site (Dec 2018 - Dec 2019)

(mean ± se)

6.4 Photographs

6.5 Subconsultants Report

"*Overview of the Ichthyoplankton sampling program in the Lower North Arm, Western Port*"
Report to CEE Pty Ltd Environmental Scientists and Engineers.
December 2019

Authors:
Dr. Gregory Jenkins
School of BioSciences, The University of Melbourne, Parkville Vic 3010

Report follows

Executive Summary

The proposed AGL Gas Import Jetty Project to be located at Crib Point, Western Port, is currently undergoing an Environment Effects Statement (EES) process. The EES will look at the potential environmental, social, economic and planning impacts and the approach to mitigating these impacts. It will give decision makers such as EPA Victoria the information they need to determine whether planning approvals should be granted and what conditions should apply.

This report presents the results of ichthyoplankton (fish eggs and larvae) sampling at a range of sites in the Lower North Arm over 12 months to characterise the ichthyoplankton community and document the temporal and spatial variability in ichthyoplankton abundance. Detailed consideration is given to species of conservation, fishing and ecological importance. The information is used to inform consideration of the impacts of entrainment by the Floating Storage and Regasification Unit (FSRU) on the ichthyoplankton community of the Lower North Arm.

The abundance of fish larvae in the Lower North Arm was strongly seasonal with highest abundances in spring – summer and low abundance in winter. The seasonal pattern for fish eggs was slightly different with highest abundance in late winter – early spring. The fish larvae were dominated by the gobies (Gobiidae), pipefish and seahorses (Syngnathidae) and the cobbler (Tetrarogidae). In comparison with southern Western Port and Port Phillip, the larval fish community in the Lower North Arm has a stronger representation of families of fish that are associated with seagrass habitat. In contrast, the relative abundance of larvae of the ecologically important Australian Anchovy and Australian Pilchard was lower than these other locations.

The main family of conservation importance was the Syngnathidae, that were the second most abundant overall. The Syngnathids were most abundant over summer, and showed a slight tendency to be more abundant near the shallow margins of the Lower North Arm. A larval Australian Grayling was collected in September, and would likely have been migrating back to freshwater after a winter period of marine residency.

Larvae of a number of families of recreational and commercial fishing importance were collected, including targeted species such as flathead and King George Whiting. Flathead larvae were collected in spring – summer while a King George Whiting post-larva was collected in October. Other families with species of fishing importance were mainly seagrass associated (e.g. Garfish, Shortfin Pike, Grass Whiting, Leatherjacket), or were from families more associated with offshore commercial fisheries (e.g. Ling, Cod).

The spatial distribution of larvae from most families was relatively evenly spread throughout the Lower North Arm. Exceptions were Cobbler and triplefin (Tripterygidae) larvae that were more abundant at the southern sampling sites, and cardinal fish (Apogonidae) larvae that were more abundant at the northern sampling sites.

Hydrodynamic modelling indicates that 0.41% of passive particles released in the Lower North Arm (0.75% for particle release near Crib Point) would be entrained into the FSRU over 28 days. This may be compared with a conservative estimate of natural larval mortality over a similar period based on northern hemisphere species of 95%, declining to 75% for larger larvae such as King George Whiting post-larvae.

The period of highest larval fish abundance coincided with the spring – summer period of lowest gas demand and therefore entrainment volume, potentially offsetting some of the impact of entrainment.

Entrainment of key species will be affected by larval behaviour, for example, King George Whiting post-larvae are mainly found near the surface during daylight, and would likely occur at a shallower depth than the FSRU intake. Larger, older larvae have significant swimming abilities, and may be able to avoid the maximum 0.15 m s^{-1} current at the mouth of the FSRU intake.

Introduction

The marine environment of Western Port is characterised by a range of habitats, including seagrass, mangroves, algae and reefs, that support a rich and diverse fish fauna (Jenkins *et al.* 2015). Species from these diverse habitats release their eggs and larvae into the water column where they mix and are dispersed by currents until they reach the early juvenile stage. The egg and larval stage in marine fish (ichthyoplankton) is characterised by high mortality rates, and the early stages are important in determining levels of recruitment and abundance of the population (Houde 2008). Spatial and temporal variability in Western Port ichthyoplankton communities are the product of life history (spawning location, spawning period, larval period), mortality (mainly predation), and dispersal related to the wind driven hydrodynamics in Bass Strait and tide dominated hydrodynamics in Western Port.

There has been commercial fishing in Western Port since the early 1900's (or earlier) (Conron *et al.* 2016). In December 2007, commercial netting methods were no longer allowed in Western Port, so that since then there has only been a very low level of commercial fishing (Conron *et al.* 2016). At the same time, the recreational fishery in Western Port is very important and represents the second-largest recreational fishery in Victoria (Conron *et al.* 2016). In addition to species of importance to fishing, there are also conservation listed fish species in Western Port, as well as ecologically important species that are key components of the food chain (Jenkins 2011).

The proposed AGL Gas Import Jetty Project to be located at Crib Point is currently undergoing an Environment Effects Statement (EES) process. The EES will look at the potential environmental, social, economic and planning impacts and the approach to mitigating these impacts. It will give decision makers such as EPA Victoria the information they need to determine whether planning approvals should be granted and what conditions should apply.

This report relates to the technical studies on ichthyoplankton (fish egg and larval) communities and will be used to inform assessment of entrainment impacts due to the Floating Storage and Regasification Unit (FSRU) operation. The studies were designed to document the spatial and temporal variability in the ichthyoplankton community of the Lower North Arm and Confluence Zone over 12 months. The data was designed to provide a basis for estimating the impacts of entrainment on ichthyoplankton populations in Lower North Arm, and implications for the Western Port ecosystem.

Entrainment occurs due to extraction of water from Western Port for use in FSRU systems including open-loop heat exchanger, closed-loop heat exchanger, engine cooling, freshwater generation and ballasting. Water extracted from Western Port during FSRU operation will be treated with chlorine to prevent biofouling within the ship's systems. Ichthyoplankton that are entrained during FSRU operation are assumed to experience 100 per cent mortality. Entrainment and mortality of plankton was identified as the key impact pathway for the marine ecosystem due to the magnitude of entrainment volumes relative to the volume and exchange characteristics of Lower North Arm, and the high value of the Western Port ecosystem.

Extraction of water from Western Port will be via one or more intakes mounted on the side of the FSRU. The distance of the intake below the surface and above the seabed will vary with tide and vessel

load but will always be in the zone from 4 m above the seabed to 1.5 m below the surface. The flow into the intake will be horizontal with a velocity of 0.15 m s^{-1} or less. The intakes will be fitted with screens with a maximum aperture of 100 mm by 100 mm.

Entrainment volumes will vary according to FSRU production rates (determined by demand for gas) and operational state (closed, open loop). Demand for gas in Victoria is projected to peak at 750 MMscfd in the coldest months of Winter (July and August). Demand is projected to be lowest in March, April and October (250 MMscfd). Demand in May, June, September and November to February is projected to be 500 MMscfd. The proposed project has a design life of 20 years but may extend for a longer period if demand for gas is maintained. Seasonal patterns in energy demand may change over this period. The entrainment volume corresponding to the maximum regasification rate in winter is estimated to be 468,000 m^{3} d^{-1}, while the annual-average entrainment volume is expected to be 312,000 m^{3} d^{-1}. Entrainment will occur at Crib Point Jetty Berth 2, in mid-water on the west side of the main channel in North Arm where the minimum water depth (LAT) is 13 metres.

Objectives

1. To undertake a sampling program designed to characterise the community structure and spatial and temporal variability of ichthyoplankton in the Lower North Arm of Western Port.

2. To use the information to inform consideration of the impacts of entrainment by the Floating Storage and Regasification Unit on the ichthyoplankton community in the Lower North Arm of Western Port.

Materials and Methods

Sampling Sites

The plankton study involved monthly sampling at 7 sites distributed in Lower North Arm and the Confluence Zone, and fortnightly sampling at Crib Point Jetty Berth 2. A map of the sites used during the monitoring program is shown in Figure 1. Sites were distributed along two transects: a north-south transect with sites distributed from the northern part of Lower North Arm to the confluence zone in the south; and a west-east transect with sites distributed between the 10 m depth contours to the west and east of Crib Point Jetty.

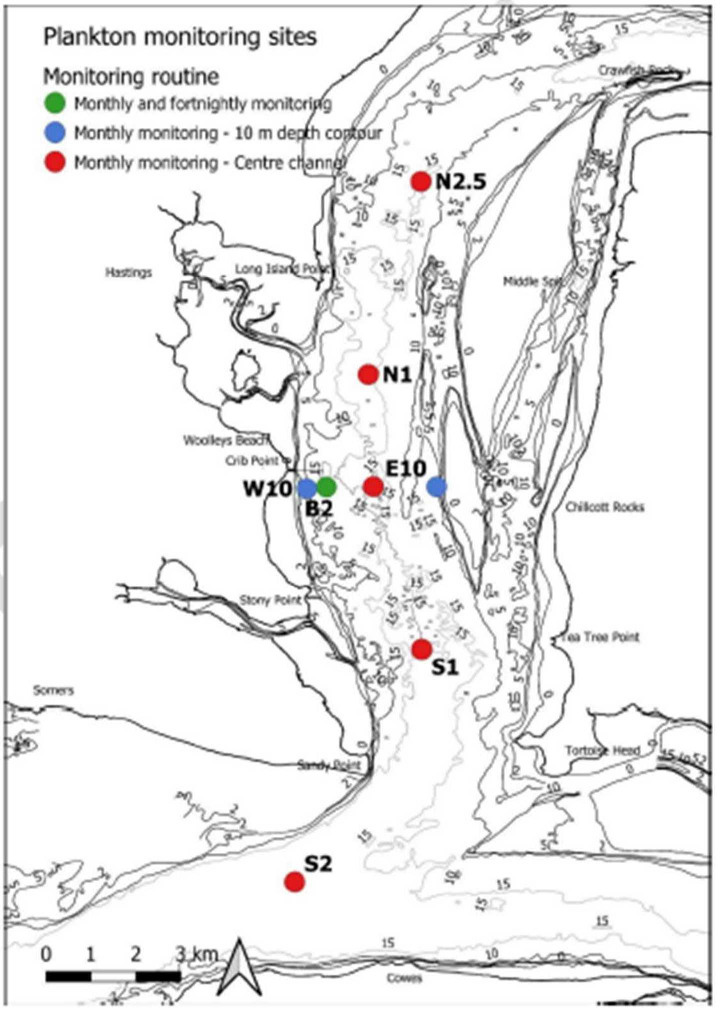

Figure 1. Ichthyoplankton sampling sites in Western Port

The north-south transect was designed to document spatial variability along the tidal gradient in Lower North Arm: waters in the south of Lower North Arm are exchanged with Bass Strait water on a shorter time-scale than those in the north of Lower North Arm. At the same time, this transect documented conditions from sites near the wave and current exposed deep channels in the Confluence Zone to sites near the extensive intertidal and shallow subtidal seagrass beds in Upper North Arm. Sites along the north-south transect were CPN2, CPN1, CPC1, CPS1 and CPS2. The location of some sites was adjusted during the program as understanding of the scale of spatial variability grew while ensuring each survey could be completed in a single day. Sites CPN2 and CPN3 showed similar plankton, water quality and hydrodynamic features and were merged into an intermediate location in March 2019, named CPN2.5 (Figure 1). Site CPS1 at the south end of Lower North Arm was expected to show more oceanic conditions, particularly at high tide, due to its proximity to the Confluence Zone and Western Entrance segments. The first six months of the monitoring program showed that conditions at CPS1 were quite similar to other Lower North Arm sites regardless of tide. Therefore, site CPS2 was added to the program in August 2019 to document conditions in the Confluence Zone where distinctly more oceanic conditions prevail. Site CPN1 is near a long-term water quality monitoring site used by EPA Victoria (Hastings) while site CPC1 is in the middle of the channel directly east of Crib Point Jetty Berth 2.

The east-west transect was designed to document spatial variability between waters over different benthic habitats in the main Lower North Arm channel. Sites on the 10 m contour east (CPE10) and west (CPW10) of Crib Point are each around 100 m from shallow subtidal and intertidal seagrass beds. These sites were of particular importance for the ichthyoplankton studies, as some fish species, including the economically important King George Whiting, seek out seagrass habitat at the end of their larval period (Jenkins, 2019). Selection of the 10 m depth contour for the east and west sites also considered the predicted extent of discharge plumes from the FSRU. The cooler-water discharge will form a mid-water or seabed plume at depths greater than 10 m. The Crib Point Berth 2 site is in the location of the proposed FSRU where entrainment will occur, and is adjacent to the artificial reef (pile) habitat created by the jetty.

Timing

Ichthyoplankton surveys at all eight monitoring sites were undertaken monthly between December 2018 and October 2019 inclusive (total of 12 surveys). Each monthly survey has been separated by a minimum of 3 and a maximum of 5 weeks. Additional plankton sampling at the Crib Point Jetty Berth 2 site (CPB2) has been undertaken every alternate fortnight (total of 26 surveys at this site).

The tides in Lower North Arm generate an average 6 km excursion over the upper layers of the water column (CEE, 2018a), that is, water moves 6 km on average with each ebb and flood tide. The north-south monitoring transect spans 16 km, or 2.7 times the tidal excursion. A single plankton survey took around 6 hours, or about the same time as an ebb or flood tide, so had a minimum effective spatial coverage of 10 km (16 km minus 6 km tidal excursion). The state of the tide was taken into account during monthly surveys to maximise the effective spatial coverage of the survey where possible. The order in which sites were sampled depended on the direction of tidal flow during the survey. For example, when sampling commenced near the end of an ebb tide, the survey commenced at the northern most site (CPN2.5) to sample water that had recently been further north. This meant that it was around high tide when the southern-most site (CPS2) was sampled, and waters there had recently been further south (towards Bass Strait). When sampling commenced near the end of a flood tide, the reverse

strategy was used: sampling commenced at CPS2, and finished at CPN2.5. Therefore, the maximum effective spatial coverage was 22 km (16 km plus 6 km tidal excursion).

Table 1 shows the dates of each monthly and fortnightly survey. All sampling for each survey was completed in one day except for the monthly surveys in February 2019 and late October 2019 which were completed over two days.

Table 1. Ichthyoplankton survey summary December 2018 – October 2019.

Date	Survey	S2	S1	B2	W10	E10	C1	N1	N2*
06/12/2018	Pilot			X					
11/12/2018	Monthly Survey 1		X	X	X	X	X		X
28/12/2018	Fortnightly Survey 1			X					
10/01/2019	Monthly Survey 2		X	X	X	X	X	X	X
22/01/2019	Fortnightly Survey 2			X					
04/02/2019	Monthly Survey 3		X	X	X	X	X		
11/02/2019	Monthly Survey 3							X	X
11/02/2019	Fortnightly Survey 3			X					
07/03/2019	Monthly Survey 4		X	X	X	X	X	X	X
20/03/2019	Fortnightly Survey 4			X					
04/04/2019	Monthly Survey 5		X	X	X	X	X	X	X
18/04/2019	Fortnightly Survey 5			X					
03/05/2019	Monthly Survey 6		X	X	X	X	X	X	X
15/05/2019	Fortnightly Survey 6			X					
07/06/2019	Monthly Survey 7		X	X	X	X	X	X	X
21/06/2019	Fortnightly Survey 7			X					
03/07/2019	Monthly Survey 8		X	X	X	X	X	X	X
18/07/2019	Fortnightly Survey 8			X					
02/08/2019	Monthly Survey 9	X	X	X	X	X	X		X
13/08/2019	Fortnightly Survey 9			X					
03/09/2019	Monthly Survey 10	X	X	X	X	X	X	X	X
18/09/2019	Fortnightly Survey 10			X					
1/10/2019	Monthly Survey 11	X	X	X	X	X	X	X	X
16/10/2019	Fortnightly Survey 11			X					
29/10/2019	Monthly Survey 12	X	X	X	X				
30/10/2019	Monthly Survey 12					X	X	X	X
Total number of surveys		3	12	20	12	12	12	10	12

X = sampled,

*Sites N2 and N3 were merged into one intermediate location in March 2019. This report uses the average for the two sites as data for N2 prior to March 2019, data for site N2 from April onwards are from the intermediate location.

Sampling Equipment and Method

Ichthyoplankton were sampled using a conical net made from 500 µm opening mesh that was attached to a rigid metal ring. Sampling in the early parts of the program used a 1 m diameter, 5 m long net, while all subsequent sampling used a 0.8 m diameter, 4 m long net. A 15 kg depressor weight was attached to the bottom of the net mouth. A 100 m long warp (rope) marked at 4 m increments was attached to a yoke on the top of the net mouth for towing. The cod end was 100 mm diameter and 200 mm long. A 1.5 kg weight was attached to the cod-end to keep it level with the rest of the net during tows. A depth logger was attached to the top of the net mouth to record sampling depth. A General Oceanics mechanical flow meter with a low velocity impeller was fixed across the mouth of the net to measure the flow of water passing through the net.

All ichthyoplankton samples were collected using a stepped-oblique tow from near the surface to near the seabed and back to the surface. This gave each tow a parabolic depth profile. The net was towed at a steady speed of 1 to 1.5 knots through the water (average of 0.6 m/s) and each tow lasted 10-15 minutes. The net was raised and lowered during the tow using the marked warp. The 4 m warp increments provided depth-steps of approximately 2 m. At the deeper centre channel sites and Crib Point Berth 2 the net was towed for two minutes at each 4 m warp increment up to 24 m. The net was towed for one minute at each increment while lowering the net, two minutes at the maximum increment, and one minute at each increment while raising the net. The total tow time included 2-3 minutes accumulated while moving between each depth increment. Centre channel and Berth 2 tows sampled ichthyoplankton from between 3 m depth and 13 m depth. The volume that passed through the net while it was between the surface and the 3 m depth increment, or below 13 m depth, was generally small relative to the remainder of the tow. At the shallower east and west 10 m contour sites the net was towed for 2.5 minutes at each 4 m warp increment.

Upon completion of each tow the sides of the net were rinsed from the outside from the top to the bottom of the net. Samples were concentrated by draining excess water through the side of the net and rinsing ichthyoplankton back into the cod-end.

One ichthyoplankton sample was collected at each site in each survey and fixed in 5% v/v buffered formalin. Clove oil was used to euthanise juvenile or post-larval fish and cephalopods where present prior to fixing in formalin.

Laboratory Processing of Samples

Preserved ichthyoplankton samples were initially sorted by CEE scientists who separated and counted all fish eggs and larvae, and cephalopods. For samples with very large numbers of fish eggs, the total number was estimated by transferring all eggs to a gridded sorting tray, counting the eggs in 10 randomly distributed grid-cells, and scaling the number up to the area of the tray.

The ichthyoplankton and cephalopods were sent to an ichthyoplankton specialist at the Australian Museum for identification and archiving. Identification was to the lowest practical level, which was typically genus or species for fish larvae, juvenile fish and cephalopods. Species for which larval characters are undescribed were differentiated into families, with lower classification into "types" based on morphological characteristics. In some cases, the species of specimens for which larvae are only described to family taxonomic level could be inferred based on the known distribution of species within the family.

Eggs of pilchards and anchovy were identified based on distinct egg morphology, but identification of the species for other fish eggs was not possible.

Results

Fish larvae were most abundant at the start of the survey in summer, declining to an order of magnitude lower abundance in late autumn-winter before increasing again in spring (Table 2). Fish eggs showed a similar seasonal pattern to larvae but abundances were higher in late winter-spring than summer (Table 2). Abundances of cephalopod larvae were highest at the start of the survey in summer and thereafter declined to low levels in winter-spring (Table 2).

Fish larvae came from 28 fish families, dominated by the Gobiidae and to a lesser extent by the Syngnathidae and Tetrarogidae (Table 2). In contrast to the diversity of families represented by larval fish, cephalopod larvae were dominated by one species, the pygmy squid, *Xipholeptos notoides*, together with a few individuals of the southern dumpling squid, *Euprymna tasmanica*.

Amongst the dominant larval fish families, the Gobiids were most abundant in summer and to a lesser extent spring, reflecting the seasonal pattern of total larvae due to their dominance (Table 2). This seasonal pattern was also shown by the Gobiesocidae (Table 2). Other families, including the Syngnathidae, Aracanidae, Monacanthidae, Apogonidae and Tetraodontidae, had highest abundances at the start of the survey in summer before declining to low levels for the remainder of the survey (Table 2). In contrast, the Tetrarogidae and Tripterygiidae were most abundant towards the end of the survey in spring (Table 2).

Larval fish from three families were potentially of conservation interest (Table 2). The Pale Mangrove Goby, *Mugilogobius platynotus*, is listed under the Victorian FFG Act (Jenkins 2019). Two goby species were identified, *Afurcagobius tamarensis* and *Gobiopterus semivestitus*, and the genus Nesogobius was also identified, together with 9 putative unidentified "types" of larval goby. The larvae of *M. platynotus*, however, cannot be separated from larvae of other similar goby species based on current knowledge (T. Miskiewiz, Pers. Comm.). The entire syngnathid family (Table 2) is listed under the EPBC Act, CITES, IUCN Red List (some), and the Victorian Fisheries Act (Jenkins 2019). The syngnathids identified were dominated by the pipefish, *Stigmatopora* sp., while seahorses, *Hippocampus* sp., were also present. The family Retropinnidae (Table 2) includes the Australian Grayling which is listed under the EPBC Act, IUCN Red List and FFG Act, as well as the smelts that are not conservation listed. One retropinnid larva was identified from site B2 in September.

Larval fish from ten families were potentially of recreational and commercial fishing interest (Table 2). Six families included species that are targeted by recreational fishers. The flatheads (Platycephalidae) occurred in summer but were most abundant in October at the end of the survey (Table 2). Specimens were collected along the length of the Lower North Arm but were most common at S1 and S2 (in the Confluence Zone). Larvae of the Shortfin Pike (Sphyraenidae) were collected in December from sites B2, E10 and S1. Barracouta (*Thrysites atun*) larvae were collected in winter-spring between N1 and S2. A small number of right-eye flounder (Rhombosoleidae) larvae were collected from May to October, most commonly along the east-west transect opposite Crib Point. A small number of garfish (Hemiramphidae) larvae were collected in February from sites N1 and N2.5. One post-larval King George Whiting (Sillaginidae) specimen was collected in October from site E10.

Other families included larvae of species that are taken as bycatch in the recreational and commercial fishery (i.e. not targeted but likely to be kept if caught). These included the genus *Meuschenia* in the

Monacanthids (e.g. Sixspine leatherjacket, *Meuschenia freycineti*) and potentially the grass whiting, *Halleta semifasciata* in the odacids, together with some species of wrasse (Labridae) (Table 2). A few larvae came from families with species more commonly caught in the offshore commercial fishery, including ling (*Genypterus sp.*) and cod (Moridae) (Table 2).

Larvae of two species that are key elements of the food chain for larger pelagic fish and seabirds were also collected. Larvae of the Australian Anchovy, *Engraulis australis*, were collected in February, May and September (Table 2). Larvae of the Australian Sardine, *Sardinops sagax*, were collected in summer and also in October (Table 2), along the length of the Lower North Arm and including the Confluence Zone.

Table 2. Summary of ichthyoplankton concentrations (average number per 1000 m³) by month

							Month						
			Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Total
Fish larvae			2164.48	746.57	308.49	66.49	43.47	18.93	20.32	12.64	18.65	236.94	425.73	344.38
Fish eggs			440.54	113.26	12.20	55.51	8.10	16.25	31.49	145.21	868.53	796.59	617.81	283.42
Cephalopod larvae			259.27	221.02	163.83	38.22	58.44	15.85	2.25	0.60	1.00	1.20	9.41	66.32
Family	Species	Common name												
Gobiidae[A]		Goby	1936.15	637.52	162.75	19.51	0.82	0.00	5.68	1.57	3.15	202.86	122.39	260.12
Syngnathidae[A]		Pipefish and seahorses	93.48	73.55	80.11	28.94	36.29	13.79	14.64	7.70	7.27	5.66	2.28	31.81
Tetrarogidae	*Gymnapistes marmoratus*	Cobbler	0.79	0.00	0.00	0.00	0.00	0.00	0.00	0.55	6.14	21.48	219.41	22.57
Aracanidae		Boxfish	68.41	14.78	2.24	0.48	0.00	0.00	0.00	0.00	0.00	0.00	0.00	7.01
Monacanthidae[B]		Leatherjacket	9.67	7.99	36.93	7.56	3.73	0.00	0.00	0.00	0.00	0.00	4.54	5.95
Tripterygiidae		Triplefin	0.89	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.29	4.14	45.67	4.39
Gobiesocidae		Clingfish	11.96	2.34	1.65	2.11	0.52	0.55	0.00	0.00	0.77	0.93	14.67	2.92
Apogonidae	*Siphamia sp.*	Cardinal fish	14.24	0.51	10.65	1.68	0.82	0.00	0.00	0.00	0.00	0.00	0.00	2.25

Tetraodontidae		Toad fish	6.88	6.32	0.00	5.48	1.30	0.00	0.00	0.57	0.00	0.00	0.00	1.79
Engraulidae[C]	*Engraulis australis*	Australian anchovy	0.00	0.00	9.63	0.00	0.00	2.79	0.00	0.00	0.00	0.44	0.00	1.06
Odacidae[B]		Weed whiting	8.36	0.42	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.56	0.75
Clupeidae[C]	*Sardinops sagax*	Australian sardine	0.45	0.91	0.58	0.00	0.00	0.00	0.00	0.00	0.00	0.00	6.71	0.70
Blenniidae		Blenny	2.46	1.22	2.17	0.24	0.00	0.00	0.00	0.00	0.00	0.00	0.56	0.55
Rhombosoleidae[B]		Right-eye flounder	0.00	0.00	0.00	0.00	0.00	0.70	0.00	0.52	0.00	0.87	2.85	0.47
Platycephalidae[B]		Flathead	0.27	0.25	0.00	0.00	0.00	0.55	0.00	0.00	0.00	0.00	3.88	0.40
Clinidae		Weed fish	3.69	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.29
Pomacentridae		Damsel fish	2.75	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.25
Sphyraenidae[B]	*Sphyraena sp.*	Shortfin pike	2.81	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.22
Gempylidae[B]	*Thrysites atun*	Barracouta	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.18	0.29	0.10	0.00	0.14
Callionymidae		Stinkfish	0.00	0.00	0.58	0.48	0.00	0.00	0.00	0.00	0.29	0.00	0.00	0.12
Hemiramphidae		Garfish	0.00	0.00	1.19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.09
Bothidae	*Lophonectes gallus*	Crested Flounder	0.00	0.00	0.00	0.00	0.00	0.55	0.00	0.00	0.00	0.00	0.56	0.09
Labridae[B]		Wrasse	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.13	0.09
Atherinidae		Hardyhead	0.99	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08
Moridae[B]		Cod	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.56	0.00	0.00	0.00	0.05

Retropinnidae[A]	*?Prototroctes maraena*	Grayling and smelt	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.46	0.00	0.05
Sillaginidae[B]	*Sillaginodes punctatus*	King George Whiting	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.53	0.04
Ophidiidae[B]	*Genypterus sp.*	Ling	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.44	0.00	0.00	0.04

A. Species of conservation significance B. Species of recreational or commercial fishing importance C. Species of ecosystem importance

Spatio-temporal patterns

The spatial distributions of total larvae (Figure 2) and the dominant gobiid larvae (

Figure 5) were relatively even across sites apart from the influence of very large numbers of goby larvae collected from site W10 in December. Fish eggs tended to be more abundant at the northern and southern sites (in spring), as well as at E10 (Figure 3). The spatial distribution of cephalopod larvae was relatively even across sites with the exception of lower abundances at W10 (Figure 4).

The spatial distribution of Syngnathid larvae was relatively even with a tendency towards higher abundances at N1 and W10 and lower abundances at S1 (Figure 6). On the cross-arm transect, Syngnathid larvae were slightly more abundant at W10 and E10 than B2 and C1 (Figure 6). Tetrarogid larvae, on the other hand, showed strong spatial variability, with most larvae coming from sites C1 and the southern sites (Figure 7). Aracanid larvae were relatively evenly distributed amongst sites with the exception of low abundances at N1 (Figure 8). Monacanthid larvae tended to show higher abundances towards the south, with highest abundance at S1 (Figure 9). Like Tetrarogid larvae, Tripterygiid larvae showed strong spatial variability, with most larvae collected from the southern sites (Figure 10). Spatial variability was also apparent for gobiesocid larvae that were mainly collected from W10 and S2 (in spring) (Figure 11), apogonid larvae that were mainly collected at the northern sites (Figure 12), and Tetraodontid larvae that were mainly collected from sites N2.5, and C1 and E10 in summer (Figure 13). Engraulid larvae were mainly collected on the east-west transect opposite Crib Point as well as at S1 (Figure 14).

The average number of fish families represented as larvae per site was relatively even but there was a strong seasonal pattern, with more families recorded in summer and spring than autumn and winter (Figure 15).

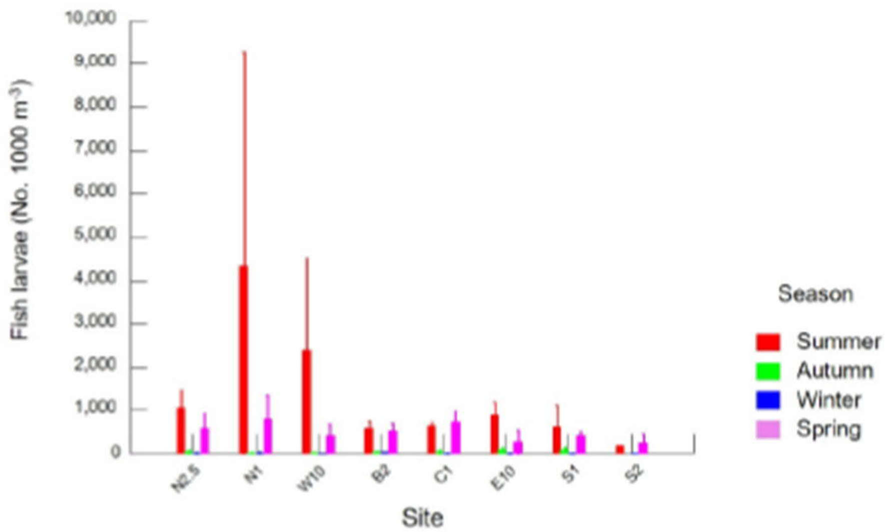

Figure 2. Average concentration of fish larvae by season and site in the Lower North Arm and Confluence Zone in Western Port. Error bars are standard error. Note: site S2 was only sampled in winter and spring.

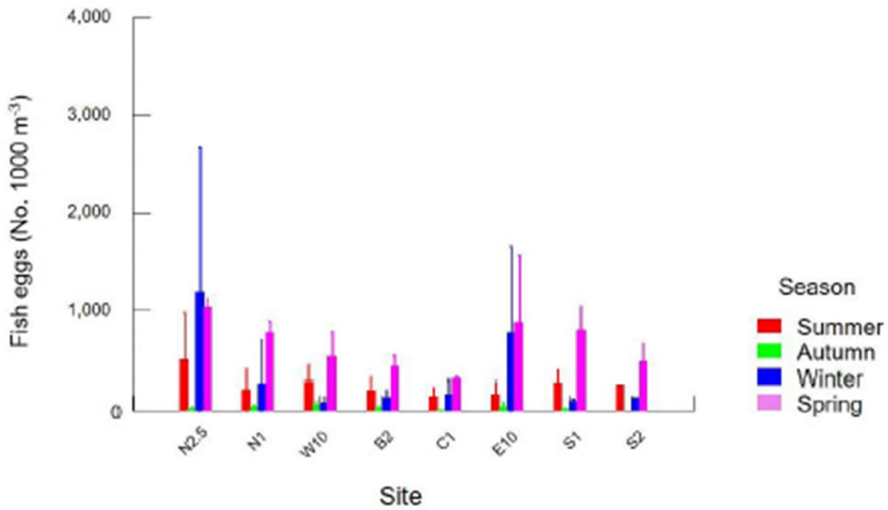

Figure 3. Average concentration of fish eggs by season and site in the Lower North Arm and Confluence Zone in Western Port. Error bars are standard error. Note: site S2 was only sampled in winter and spring.

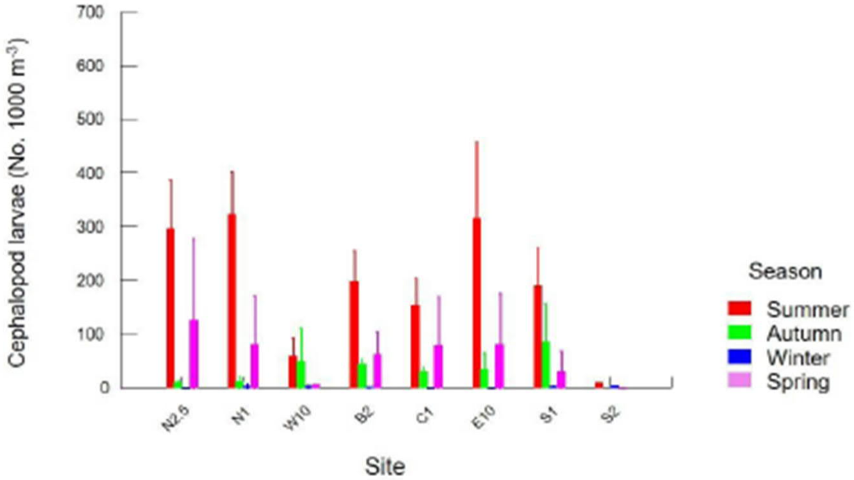

Figure 4. Average concentration of cephalopod larvae by season and site in the Lower North Arm and Confluence Zone in Western Port. Error bars are standard error. Note: site S2 was only sampled in winter and spring.

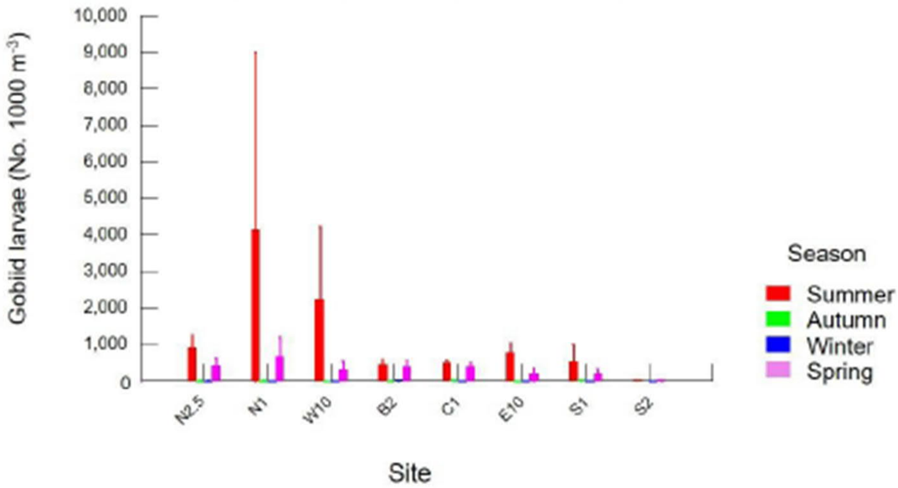

Figure 5. Average concentration of gobiid larvae by season and site in the Lower North Arm and Confluence Zone in Western Port. Error bars are standard error. Note: site S2 was only sampled in winter and spring.

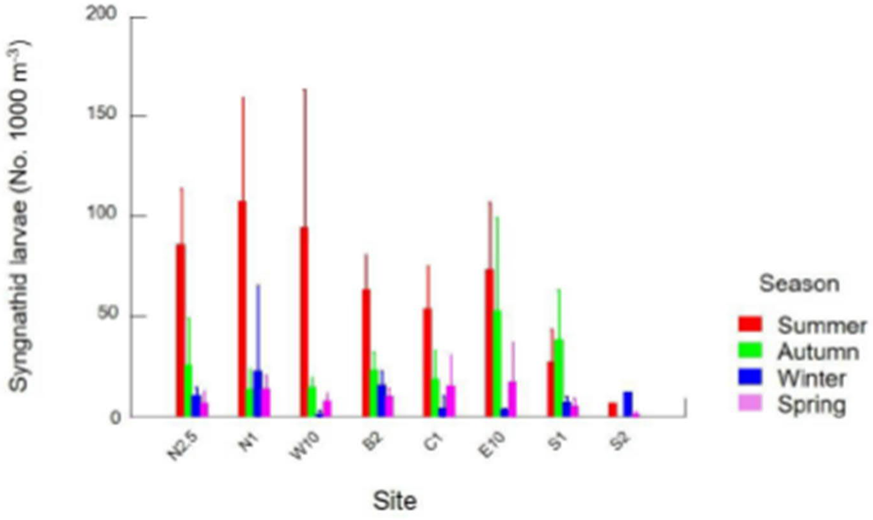

Figure 6. Average concentration of syngnathid larvae by season and site in the Lower North Arm and Confluence Zone in Western Port. Error bars are standard error. Note: site S2 was only sampled in winter and spring.

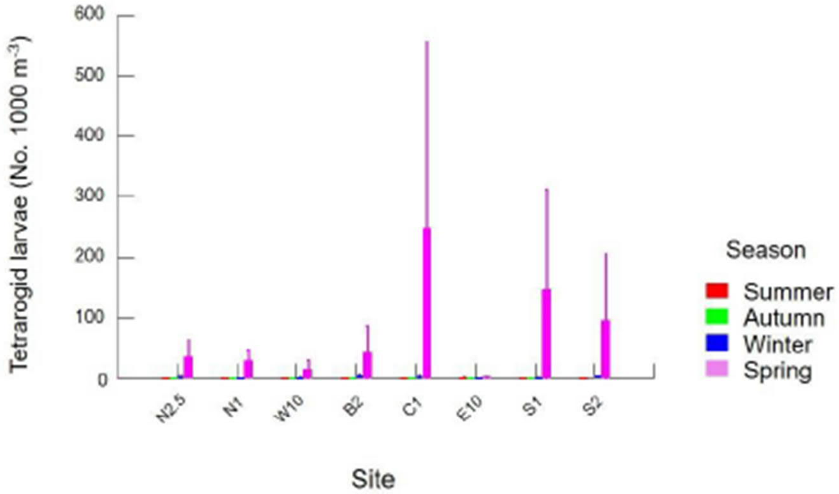

Figure 7. Average concentration of tetrarogid larvae by season and site in the Lower North Arm and Confluence Zone in Western Port. Error bars are standard error. Note: site S2 was only sampled in winter and spring.

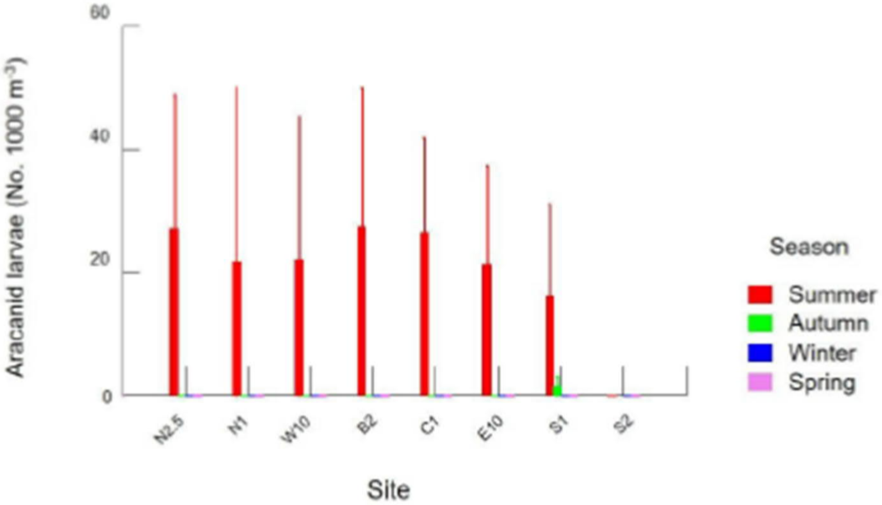

Figure 8. Average concentration of aracanid larvae by season and site in the Lower North Arm and Confluence Zone in Western Port. Error bars are standard error. Note: site S2 was only sampled in winter and spring.

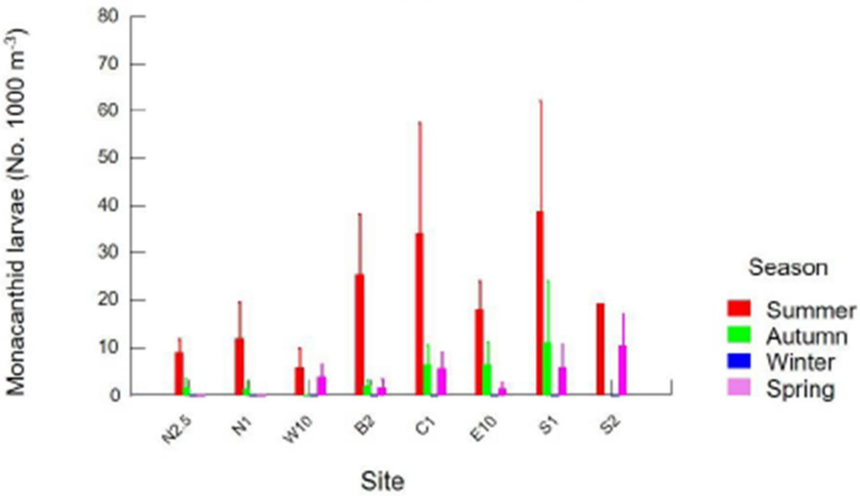

Figure 9. Average concentration of monacanthid larvae by season and site in the Lower North Arm and Confluence Zone in Western Port. Error bars are standard error. Note: site S2 was only sampled in winter and spring.

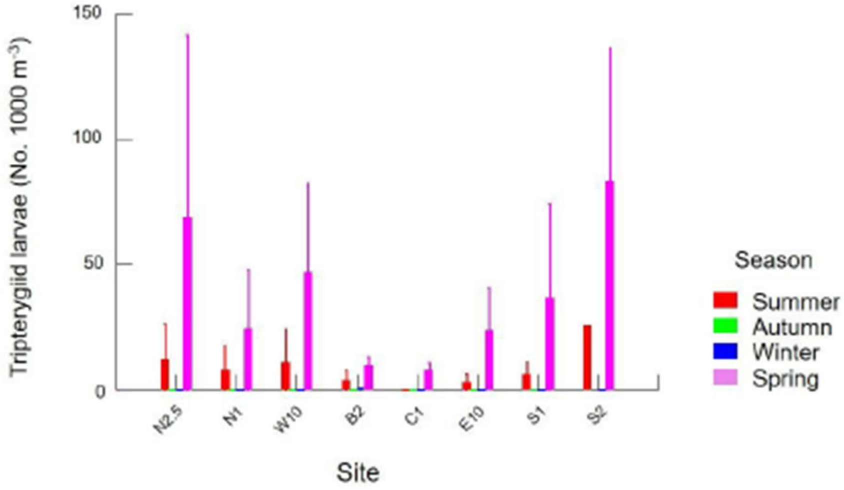

Figure 10. Average concentration of tripterygiid larvae by season and site in the Lower North Arm and Confluence Zone in Western Port. Error bars are standard error. Note: site S2 was only sampled in winter and spring.

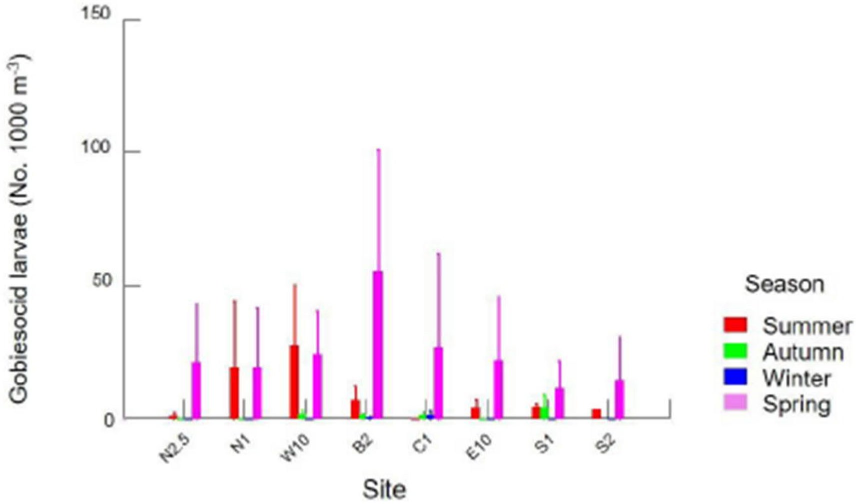

Figure 11. Average concentration of gobiesocid larvae by season and site in the Lower North Arm and Confluence Zone in Western Port. Error bars are standard error. Note: site S2 was only sampled in winter and spring.

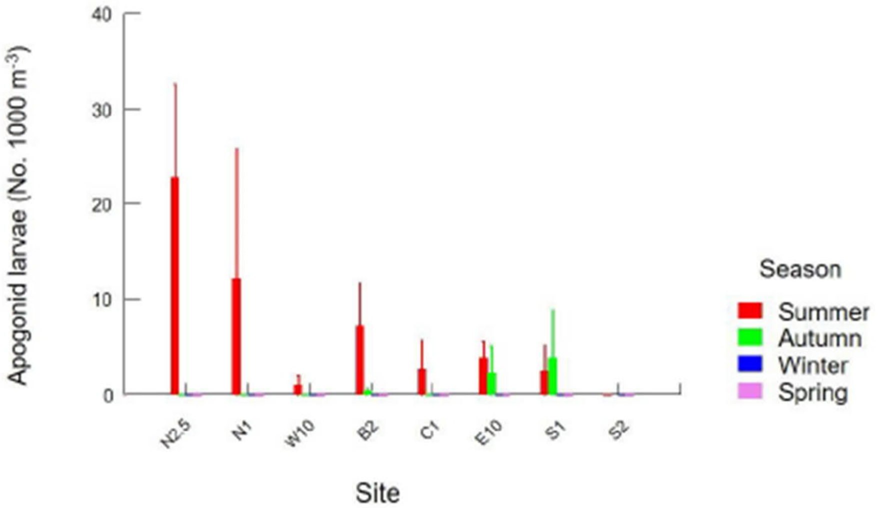

Figure 12. Average concentration of apogonid larvae by season and site in the Lower North Arm and Confluence Zone in Western Port. Error bars are standard error. Note: site S2 was only sampled in winter and spring.

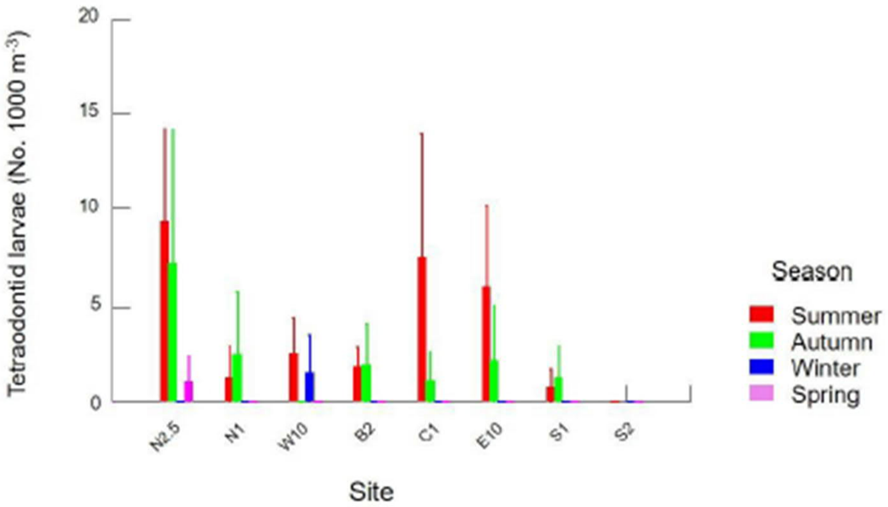

Figure 13. Average concentration of tetradontid larvae by season and site in the Lower North Arm and Confluence Zone in Western Port. Error bars are standard error. Note: site S2 was only sampled in winter and spring.

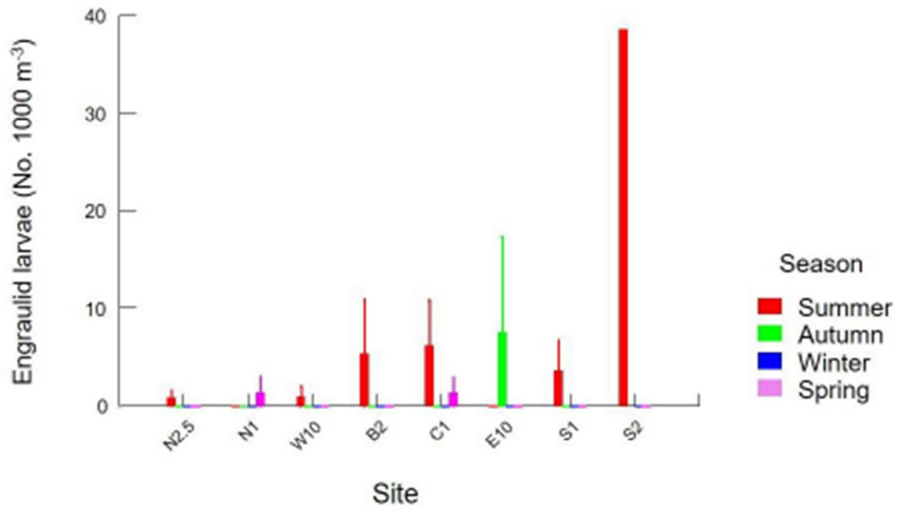

Figure 14. Average concentration of engraulid larvae by season and site in the Lower North Arm and Confluence Zone in Western Port. Error bars are standard error. Note: site S2 was only sampled in winter and spring.

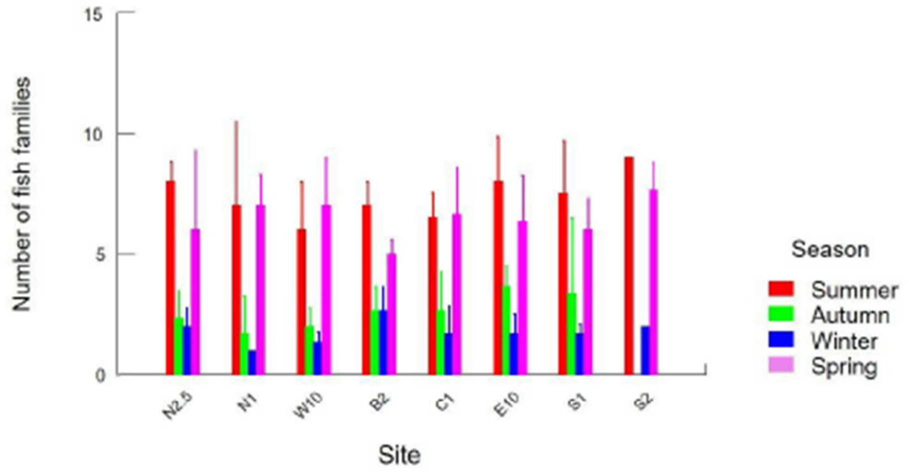

Figure 15. Average number of fish families represented as larvae by season and site in the Lower North Arm and Confluence Zone in Western Port. Error bars are standard error. Note: site S2 was only sampled in winter and spring.

Discussion

General Patterns

The abundance of fish larvae in the Lower North Arm was strongly seasonal, with highest abundances in summer and spring and low abundance in autumn and winter. This pattern was largely a reflection of the seasonal pattern of abundance of the two dominant larval fish families, the gobies and the syngnathids, as well as a number of less abundant families. The seasonal abundance of fish eggs showed a different pattern, with highest abundances in late winter – early spring. This difference between seasonal patterns of abundance for larvae and eggs is a reflection of the life histories of the dominant families. The gobies lay benthic eggs and larvae hatch and enter the water column, while in the syngnathids the males brood the eggs before larvae are released into the water column. The most abundant family that has the most common reproductive pattern for marine fish, spawning of pelagic eggs into the water column that hatch into pelagic larvae, was the Tetrarogidae, represented by a single species, the cobbler *Gymnapistes marmoratus*. Larvae of this species were most abundant in October and it is likely that the peak in egg abundance in August and September was largely a reflection of the spawning of this species.

The dominant families represented as larvae in the Lower North Arm showed consistencies but also marked differences to that seen in Port Phillip Bay. Like the current study, the dominant larvae in Port Phillip were the gobies (Jenkins 1986; Neira and Sporcic 2002). This likely reflects the high abundance and species diversity of these small benthic fish, as well as their ubiquitous habitat use (Jenkins 2019). Unlike the current study, the co-dominant or second most abundant larvae in Port Phillip were Australian Anchovy larvae, compared with 10th most abundant family in the lower North Arm. This reflects the fact that significant anchovy spawning occurs in the north of Port Phillip (Jenkins and Hatton 2007), while in contrast eggs and larvae of anchovy mostly occurred in southern Western Port and the adjacent coastal waters of Bass Strait (Hoedt and Dimmlich 1995). A major difference between the current results and previous studies from Port Phillip was the finding that syngnathids were the second most abundant family of larvae in the Lower North Arm, whereas they were more lowly ranked in the Port Phillip Bay studies (Jenkins 1986; Neira and Sporcic 2002). Larvae of the cobbler were abundant in both the Lower North Arm and in Port Phillip in late winter – spring (Jenkins 1986; Neira and Sporcic 2002).

The major difference in the fish community represented as larvae in the current study compared with previous studies in Port Phillip was the dominance of families of seagrass associated fish represented as larvae in the Lower North Arm, including the Syngnathidae, Tetrarogidae, Monacanthidae, Gobiesocidae, Apogonidae, Tetraodontidae, and Odacidae. In contrast, only the Tetrarogidae, Monacanthidae and Odacidae were ranked highly in larval abundance in Port Phillip (Jenkins 1986; Neira and Sporcic 2002). Larvae of one family of seagrass associated fish, the weedfish (Clinidae) was were common in Port Phillip (Neira and Sporcic 2002) than the Lower North Arm.

Previous ichthyoplankton sampling in southern Western Port was conducted at sites north of Phillip Island, in the Rhyll Basin and inside the Eastern Entrance (Acevedo *et al.* 2010; Kent *et al.* 2013). Like the Lower North Arm, gobies were the most abundant larvae, but there were also marked differences. The second most abundant larvae in southern Western Port were the triplefins (Tripterygiidae) (Acevedo *et al.* 2010; Kent *et al.* 2013) which were common but more lowly ranked in the Lower North Arm. Tripterygiid larvae in the current study were most abundant at the southern sites, consistent with a higher abundance of the family in southern Western Port, possibility reflecting the larger amount of reef habitat this group is primarily associated with. Abundances of other families also reflected the

proximity of sites to Bass Strait in the earlier study, with a relatively higher abundance anchovy larvae, and a relatively lower abundance of syngnathid larvae (Acevedo *et al.* 2010; Kent *et al.* 2013).

Species of Conservation Importance

As mentioned above the relative abundance of syngnathid larvae is higher in the Lower North Arm compared with southern Western Port or Port Phillip, reflecting the high coverage of seagrass in the area (Jenkins 2019). The syngnathids collected would have included older juveniles and adults associated with detached drifting seagrass as well as larvae. The pattern of higher abundance of syngnathids close to shore on the cross-shelf transect also likely reflects the fact that seagrass coverage is greatest on the shallow margins of the Lower North Arm.

Larvae of the Pale Mangrove Goby could not be separated from other unidentified goby larvae, and would be expected to be relatively rare compared to larvae of most species in the area. Western Port has the only records of this species in Victoria, with most records coming from mangrove habitat in the Lower North Arm (Jenkins 2019). Consistent with the predominant summer occurrence of goby larvae in this study, Pale Mangrove Goby are thought to spawn from November through to February in Western Port (Raadik and Hindell 2008).

One Retropinnid larva was collected from site B2 in September. This family includes the Australian Grayling and the smelts, but this specimen was almost certainly an Australian Grayling because the smelts are not known to have a marine life history phase. A period of marine residency for larvae and young juveniles of Australian Grayling from the Bunyip River has been confirmed by otolith chemistry (Crook *et al.* 2006). The most likely life-history model is that larvae drift downstream into Western Port, or possibly offshore, from April to July with a peak in May (Koster and Dawson 2010; Koster *et al.* 2013; 2018) and return upstream as young juveniles from September to December (Crook *et al.* 2006; Koster *et al.* 2019). It is therefore possible that larvae and/or young juveniles may occur in the Lower North Arm from late autumn to early summer.

Species of Recreational and Commercial Fishing Importance

The most abundant families represented as larvae that potentially included species of fishery importance were the Monacanthidae (Leatherjackets) and the Odacidae (Weed Whiting). These are seagrass associated fish that are not generally targeted by recreational fishers but may be retained if caught (bycatch). The most targeted families for recreational fishing represented as larvae were flathead and King George Whiting.

There are three main flathead species in Western Port: Sand Flathead, *Platycephalus bassensis*; Yank Flathead, *Platycephalus speculator*; and, Rock Flathead, *Platycephalus laevigatus*. Juvenile and adult Sand and Yank Flathead in Western Port are found in unvegetated silt-sand habitats (Edgar and Shaw 1995; Conron *et al.* 2016), while Rock Flathead are found on sandy habitat as small juveniles but move into seagrass habitat as adults (Edgar and Shaw 1995). Flathead larvae in the Lower North Arm were collected in late spring and summer which is consistent with ichthyoplankton sampling results from Port Phillip (Jenkins 1986; Neira and Sporcic 2002). Ichthyoplankton sampling in the south-east of Western Port recorded the presence of flathead larvae from October to March (Kent *et al.* 2013).

King George Whiting do not occur in Western Port as small larvae like most species, but rather as post-larvae that enter the bay from September to November when they are 3 to 5 months old and 15 to 20 mm in length (Jenkins *et al.* 2000; 2016). This is related to the fact that the spawning area for Whiting in central Victoria is likely to be in coastal waters of Western Victoria and south-eastern South

Australia (Jenkins *et al.* 2000; 2016). Whiting post-larvae have been collected in ichthyoplankton samples from Port Phillip from September to November (Jenkins *et al.* 1999), and from south-east Western Port in September (Kent *et al.* 2013).

Larvae of less targeted recreational fishing species such as pike, flounder and garfish were collected in low numbers and are likely to spawn within Western Port. A small number of families represented as larvae, such as the Ophiidae (Lings) and the Moridae (Cods) were collected that are more associated with offshore commercial fisheries, and it is likely that larvae would have been dispersed into Western Port from coastal spawning grounds.

Species of Ecological Importance

The Australian Anchovy and Australian Pilchard are a key food chain species supporting pelagic fish species (Hoedt and Dimmlich 1994) and seabirds, including Little Penguins (Dann 2011). As mentioned above, abundances of larvae of these species were relatively low in the Lower North Arm, which is consistent with previous sampling showing that eggs and larvae of these species are most abundant in southern Western Port and coastal waters adjacent to Western Port (Hoedt and Dimmlich 1995; Kent *et al.* 2013), indicating that most spawning occurs in these areas.

Egg and Larval Mortality

Most marine fish are highly fecund, and mortality rates are size-dependent, with the highest mortality rates occurring in the youngest stages (Peterson and Wroblewski 1984). Mortality rates of marine larvae are most commonly estimated using the "catch curve" method that quantifies the decrease in larval abundance with increasing age (or length as a proxy for age) from ichthyoplankton samples (Pepin 2016). Estimates based on species in the northern hemisphere are highly variable, but for typical larvae of approximately 5 mm in length (i.e. most of the larvae collected in this survey), the mortality rates is between ~ 10 and 60% per day (Bailey and Houde 1989; Houde 2008). For larvae of equivalent length to King George Whiting post-larvae of approximately 15 – 20 mm in length, estimates range from ~ 5 to 30 % per day (Bailey and Houde 1989; Houde 2008). In the case of larvae of pipefish, *Stigmatopora* sp., that are released into the water column at a length of approximately 30 mm, the expected mortality rate of larvae of this length would be in the order of 5% per day (Bailey and Houde 1989; Houde 2008). Unfortunately, there are no estimates of larval mortality for fish species in Western Port.

Implications of the Gas Import Jetty Project

Hydrodynamic modelling studies have shown that for passive particles released in the main channel close to Crib Point, the entrainment rate was 0.75% of particles over 28 days, and the average entrainment rate for particles released in 5 sectors throughout the Lower North Arm was 0.41% of particles over 28 days (Hydronumerics 2019). Many fish species have a pelagic larval duration in the order of one month, so this is a relevant time scale to consider the effects of entrainment. If we consider a conservative estimate of mortality over the larval phase (2 – 10 mm length) of 10% per day (see above), then approximately 95% would die over the course of 28 days. Thus, the additional mortality from entrainment into the FSRU would be very small compared to the overall level of mortality. King George Whiting post-larvae are larger (15-20 mm) and a lower conservative level of mortality of 5% per day could be assumed. In this case, approximately 75% of King George Whiting post-larvae would be expected to die over 28 days, so that the additional impact of the FSRU is slightly greater than for smaller larvae, but still very low. The same analysis would be relevant to pipefish, *Stigmatopora* sp. larvae which could also be assumed to have a mortality rate of 5% per day based on size at release into the water column. There is, of course, considerable uncertainty in these estimates, because there is wide

variation in the estimated mortality at a given size/age amongst species, and as mortality rates of larvae have not been directly measured for any species in Western Port.

The hydrodynamic modelling study assumes that larvae are dispersed passively by currents. The analysis of spatial distributions of larvae in this study tend to support this assumption, with relatively even spatial distributions of larvae from most families throughout the Lower North Arm. There are some exceptions, for example, the data suggests that syngnathid larvae are more abundant at the edges of the main channel (W10 and E10), than within the channel (B2 and C1), which may reduce the impact of entrainment at the FSRU slightly. This cross-channel pattern is likely due to the release of syngnathid larvae mainly occurring from seagrass beds along the shallower margins of the Lower North Arm.

Larval behaviour can alter passive dispersal and affect the assumptions of the modelling, as well as giving options for reducing the impacts of entrainment. For example, King George Whiting post-larvae are known to show strong diurnal vertical migration behaviour; during daylight they are concentrated near the water surface but at night they are randomly dispersed through the water column (Jenkins *et al.* 1998). Because the intake pipe of the FSRU will be at least 1.5 metres deep, it is less likely that Whiting post-larvae will be entrained during daylight hours compared to night. The fact that the ichthyoplankton net in this study did not fish in the top 3 metres and the sampling was in daylight may explain why only one King George Whiting post-larva was collected. As well as vertical migration, Whiting post-larvae, like most older stage larvae, are capable of horizontal swimming that can alter passive dispersal. In Port Phillip Bay, Whiting post-larvae were found to occur slightly closer to shore than predicted by modelling passive dispersal, most likely due to directed swimming (Jenkins *et al.* 1999). In terms of the depth of the FSRU intake, it should also be noted that pelagic fish eggs tend to be positively buoyant and will tend to accumulate near the surface, potentially reducing the rate of entrainment, although this depends on the strength of turbulent mixing.

The swimming ability of larger larvae opens the possibility that they may be able to avoid the intake of the FSRU depending on the intake current speed. The project specifications allow for a maximum intake current of 0.15 m s^{-1} at the grill. The sustained swimming speed that King George Whiting post-larvae can maintain for 2 hours is only 0.06 m s^{-1} (Jenkins and Welsford 2002), however the burst speed they are capable of to avoid danger such as predators is unknown. Using a measure of swimming speed where the current in a swimming chamber was increased by 0.02 m s^{-1} in 5 minute intervals, larvae of temperate fish species were found to increase rapidly in swimming ability from hatching to the end of the larval stage near settlement (Clark *et al.* 2005). The larger pre-settlement larvae were capable of swimming at speeds of 0.15 to 0.2 m s^{-1} under this measure (Clark *et al.* 2005). Thus, larger, older fish larvae may be able to avoid the FSRU intake by active swimming.

In terms of entrainment overall, the impact will be highest over spring and summer given the strong seasonal pattern in ichthyoplankton abundance. Spring and Summer also coincides with the period when larvae of most of the conservation, fishing and ecologically important species are in the water column. The period of highest larval abundance coincides with the period of lower gas demand, potentially offsetting some of the impact of entrainment.

The main species of importance that was collected in the area of the proposed FSRU over the winter-early spring period was the Australian Grayling. Larvae drift downstream and enter Western Port from April to July with a peak in May (Koster and Dawson 2010; Koster *et al.* 2013; 2018). Larvae then undergo a period of marine residency before returning upstream as young juveniles from September to December (Crook *et al.* 2006; Koster *et al.* 2019). It is not known whether larvae remain in Western

Port or are dispersed offshore over the period of marine residency (Crook *et al.* 2006). The single larva collected in this study in September was likely returning to freshwater habitat.

Summary

The abundance of fish larvae in the Lower North Arm was strongly seasonal with highest abundances in spring – summer and low abundance in winter. The seasonal pattern for fish eggs was slightly different with highest abundance in late winter – early spring. The fish larvae were dominated by the gobies (Gobiidae), pipefish and seahorses (Syngnathidae) and the cobbler (Tetrarogidae). In comparison with southern Western Port and Port Phillip, the larval fish community in the Lower North Arm has a stronger representation of families of fish that are associated with seagrass habitat. In contrast, the relative abundance of larvae of the ecologically important Australian Anchovy and Australian Pilchard was lower than these other locations.

The main family of conservation importance was the Syngnathidae, that were the second most abundant overall. The Syngnathids were most abundant over summer, and showed a slight tendency to be more abundant near the shallow margins of the Lower North Arm. A larval Australian Grayling was collected in September, and would likely have been migrating back to freshwater after a winter period of marine residency.

Larvae of a number of families of recreational and commercial fishing importance were collected, including targeted species such as flathead and King George Whiting. Flathead larvae were collected in spring – summer while a King George Whiting post-larva was collected in October. Other families with species of fishing importance were mainly seagrass associated (e.g. Garfish, Shortfin Pike, Grass Whiting, Leatherjacket), or were from families more associated with offshore commercial fisheries (e.g. Ling, Cod).

The spatial distribution of larvae from most families was relatively evenly spread throughout the Lower North Arm. Exceptions were Cobbler and triplefin (Tripterygidae) larvae that were more abundant at the southern sampling sites, and cardinal fish (Apogonidae) larvae that were more abundant at the northern sampling sites.

Hydrodynamic modelling indicates that 0.41% of passive particles released in the Lower North Arm (0.75% for particle release near Crib Point) would be entrained into the FSRU over 28 days. This may be compared with a conservative estimate of natural larval mortality over a similar period based on northern hemisphere species of 95%, declining to 75% for larger larvae such as King George Whiting post-larvae.

The period of highest larval fish abundance coincided with the spring – summer period of lowest gas demand and therefore entrainment volume, potentially offsetting some of the impact of entrainment. Entrainment of key species will be affected by larval behaviour, for example, King George Whiting post-larvae are mainly found near the surface during daylight, and would likely occur at a shallower depth than the FSRU intake. Larger, older larvae have significant swimming abilities, and may be able to avoid the maximum 0.15 m s^{-1} current at the mouth of the FSRU intake.

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