University of Essex

Quantification and staging of oyster larvae

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Summary

Colchester Oyster Fishery (COF) required assistance with counting of oyster larvae and was interested in quantifying the absolute abundance of *Crassostrea gigas* larvae in the Colne estuary and *Ostrea edulis* larvae in land-based rearing ponds. Zooplankton sampling, water collection for chlorophyll-*a* (chl-*a*) quantification and continuous water temperature measurements were conducted in 2016 and are currently continued from mid-July 2017. The appearance of larvae (spawning) in the estuary followed an 11 day period of increasing water temperatures in the second half of July. No oyster larvae were detected in the Western and Eastern Ponds.

Background

Oysters release free-swimming larvae that, depending on temperature and food availability, feed for about 14 days in the plankton before the last planktonic stage (pediveliger stage) settles onto suitable surfaces (Figure 1).

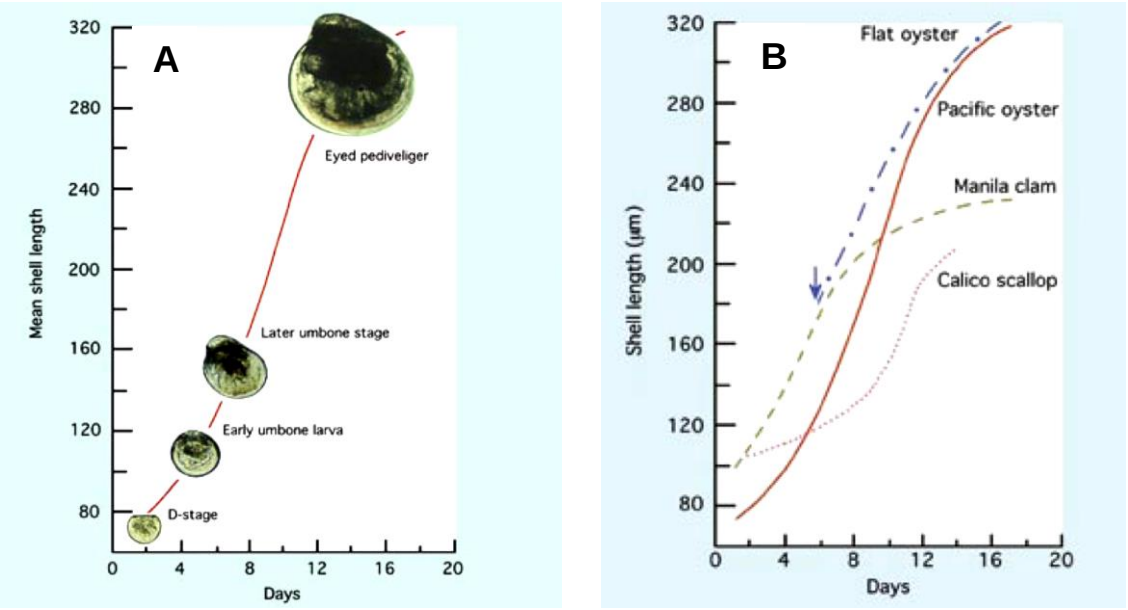


Figure 1. (A) Photomicrographs of the growth and development of Pacific oyster, *Crassostrea gigas* from release of the free-swimming larva (planktonic D-stage) to its settlement (spatfall). **(B)** Comparative growth of planktonic larvae of four bivalve species (European flat oyster, *Ostrea edulis*; Pacific oyster, *Crassostrea gigas*; Manila clam, *Tapes philippinarum*; Calico scallop, *Argopecten gibbus*) from the D-stage larva to settlement and metamorphosis when cultured at 24 ± 2 °C. Day 0 denotes the day that eggs were fertilized. The blue arrow shows the day that flat oyster larvae were released by the brooding female (adapted from Helm et al. (2004)).

Oysters show gregarious spat-fall, hence they preferentially settle where other oysters, or oyster and other bivalve shells (cultch) are present. This knowledge can be used to manage oyster populations and increase annual harvest. The Colchester Oyster Fishery (COF) is

interested in optimising the timing for laying cultch for larval attachment and increasing the harvest of *Crassostrea gigas* and, possibly, the superior *Ostrea edulis* in the future.

This study aims to collect information on the seasonal abundance of planktonic oyster larvae in the Colne Estuary and rearing ponds on the premises of COF in East Mersea. Results for larvae abundance were compared with data on water temperature and phytoplankton biomass, and the vertical distribution of temperature and phytoplankton pigments including chlorophyll-*a* (chl-*a*) were quantified to assess levels of mixing and biodiversity.

Approach

Sampling and plankton identification were conducted and overseen by Dr Michael Steinke and training provided to a member of staff at COF (Mr Alex Luscombe) and a research assistant at the University of Essex (Ms Luli Randell) to conduct water and plankton sampling autonomously during the second half of the study. Water and plankton samples were collected on 14 occasions from the area known as the Binnaker (B; approximately 51.8 N, 1.0 E) and from the rearing ponds located on site at COF (Western Pond = W; Eastern Pond = E; Table 1). Sampling at the Binnaker was conducted close to the time of high tide slack water using the COF boat, and sampling at Western and Eastern Ponds was conducted using an inflatable dinghy. Two temperature data loggers (HOBO UA-002-64 temperature/light logger, Tempcon Instrumentation Ltd., Arundel, UK) were placed submerged on a weighted line connected to a buoy at the Binnaker (22 April to 1 November 2016) and Eastern Pond (13 July to 12 September 2016). These continuously recorded water temperature at 5 min intervals with the exception of 15 to 29 June when no data logger was deployed at the Binnaker. Mean Daily Water Temperature (MDWT), the mean temperature over a 24-h day, and a running mean using a 24-h window were calculated.

At each location, two plankton samples were taken using vertical tows with a 300 mm diameter plankton net (mesh size of 53 µm) that was fitted with 350 mL collection bottles at the cod-end. Plankton samples were stored in a cooler box in the dark for transport to the laboratory at the University of Essex. Upon arrival, plankton were distributed evenly by 10 slow 'head-over-heel' rotations of the sample bottle. One plankton sample was immediately preserved by filling 210 mL of homogenised plankton sample into a brown medical flat and adding 5 mL of Lugol's iodine solution before storage at 4 °C. The other plankton sample was used for live observations in several sub-aliquots using a dissecting microscope (Olympus SZX2). Counting and photographic sample documentation were achieved after de-staining 8 mL of Lugol's-preserved sample with 2 mL of a 5% Na-thiosulfate solution and microscopic visualisation of plankton (Leica DMI6000B automated inverted microscope with Leica Application Suite software version 4.6.0).

A water sample was taken from just below the surface at each location for the determination of chl-*a* concentration as a measure of phytoplankton biomass. The water sample was stored in a cooler box in the dark for transport to the laboratory at the University of Essex where an aliquot of the water sample (100 to 300 mL depending on amount of suspended material) were filtered through a glassfibre filter (Whatman GF/F, 47 mm diameter) that was placed into an individual centrifugation tube and immediately stored at -20 °C for later assessment of phytoplankton biomass using chl-*a* extraction in methanol at 4 °C and

spectrophotometric quantification after the methods described in Ritchie (2008). An aliquot of the filtrate was used to determine salinity using a hand-held refractometer.

Table 1. Sampling dates, locations, times, times of high tide and mean daily water temperatures. ND, no data.

Date in 2016	Location*	Time of sampling	Time of High Tide	Mean daily water temperature [°C]
10-Jun	B1	16:40	16:49	18.3
	W1	16:15		ND
23-Jun	B2	15:30	14:59	ND
	W2	16:00		ND
	E2	16:07		ND
04-Jul	B3	13:25	12:47	16.8
	W3	12:55		ND
14-Jul	B4	08:35	08:12	17.0
	E4	09:20		21.7
20-Jul	B5	13:37	13:23	21.2
	E5	14:35		26.6
28-Jul	B6	07:13	07:06	22.2
	E6	07:41		23.9
04-Aug	B7	13:44	14:04	20.2
	E7	13:18		22.0
16-Aug	B8	ND	11:32	19.8
	E8	ND		22.0
18-Aug	B9	ND	13:01	20.8
	E9	ND		22.4
23-Aug	B10	ND	16:25	21.4
	E10	ND		22.2
25-Aug	B11	ND	18:02	23.0
	E11	ND		23.8
30-Aug	B11b	ND	11:36	21.4
	E11b	ND		23.5
05-Sep	B12	15:00	15:22	18.5
	E12	14:00		21.4
12-Sep	B13	08:45	08:43	19.8
	E13	14:20		ND

* B = Binnaker, W = western pond, E = eastern pond (drained and refilled with seawater on 8-9 July 2016)

On 20 July 2016 a fluorescence probe (FluoroProbe, bbe Moldaenke, Schwentinental, Germany) was used to characterise the vertical distribution of temperature, chl-*a* and other phytoplankton pigments to characterise the biodiversity by distinguishing between four different taxonomic groups including (i) chromophytes (= diatoms, dinoflagellates and chrysophytes), (ii) chlorophytes (green algae), (iii) cryptophytes, and (iv) cyanobacteria (blue-green algae).

Results and Discussion

Water temperature: Seasonally-increasing water temperature is critical for the initiation of spawning in bivalves with a basal threshold temperature of 20 °C reported for *C. virginica* (Nelson 1928). Once this basal water temperature is reached, spawning occurs after pulses of 2 to 5 °C increases in water temperature (Bernard et al. 2016). Water temperature at the Binnaker (Figure 2) demonstrated the expected seasonal pattern with relatively low MDWT at the start of the study (23 April; 10.6 °C), maximum MDWT in July (24 July, 24.8 °C) and lower MDWT at the end of sampling (12 September, 19.8 °C). MDWT crossed the 20 °C threshold on 19 July (20.7 °C) during a 10-day period of rapid rise in water temperature

(average increase of 0.8 ± 0.48 ºC per day) from 14 to 24 July. Water temperature was above the 24 °C threshold on 22-25 July (24.1 to 24.8 °C). Daily fluctuations in water temperature (0.5 to 5.8 °C) were influenced by the tidal flow typically with a flood tide on average 2.1 °C warmer than the ebb tide.

Water temperature at Eastern Pond was monitored from 13 July to 12 September and showed similar trends during that period to the Binnaker with temperatures ranging from a minimum MDWT of 20.7 ºC on 11 August to a maximum of 26.6 ºC on 20 July. MDWT was on average 2.5 ± 1.16 ºC higher (range 0.4 to 5.5 ºC) than at the Binnaker and lacked the tidally-driven daily fluctuations (Figure 3). It is likely that the lack of flushing (stagnant pond), relatively small water volume and the black lining contributed to the increased internal heating.

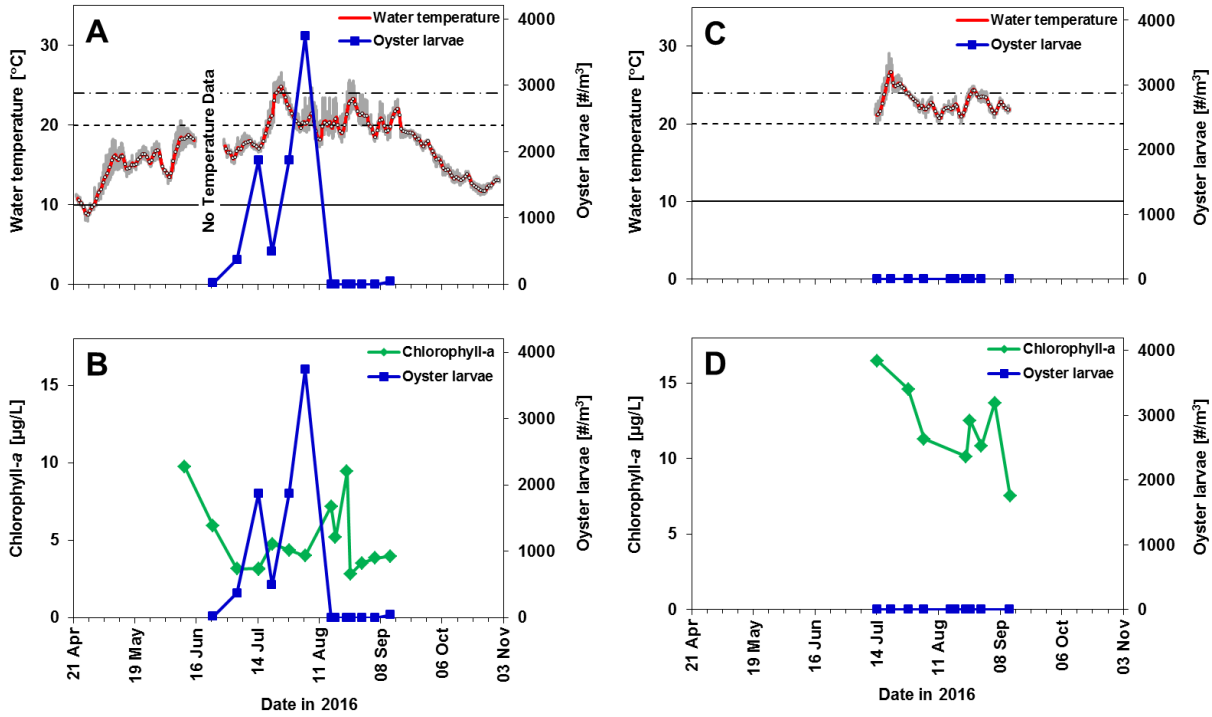


Figure 2. Abundance of oyster larvae, water temperature (A, C) and chlorophyll-*a* (B, D) at the Binnaker (left panels) and Eastern Pond (right panels). Presented are the abundance of oyster larvae on dates shown in Table 1 (blue squares). Red line indicates 24h running average of water temperature, grey area indicates range of temperature data and white circles show Mean Daily Water Temperature (MDWT). Note that no temperature data were collected at the Binnaker on 15-28 June 2016. Black line, dashed line and dash-dotted lines indicate water temperatures of 10, 20 and 24 °C, respectively.

Phytoplankton (chl-a) biomass: Samples for the quantification of chl-*a* were taken in parallel with plankton samples for the enumeration of larvae density. At the Binnaker, concentrations were higher at the start of the study (9.7 µg chl-*a*/L on 10 June) that steadily decreased to lower values of 3.9 µg chl-*a*/L on 14 June to 4 August in agreement with the lower abundance of diatoms during this period (see Appendix A). Eastern Pond showed higher chl-*a* concentrations ranging from a maximum of 16.5 µg chl-*a*/L on 14 July to 7.5 µg chl-*a*/L on 12 September.

Fluorescence characteristics: Data from the fluorescence probe indicated that the estuary (Binnaker; sample B5 on 20 July) was well mixed with little vertical structure in either the temperature (Fig. 2A) or pigment data (Fig. 2B). Temperature ranged from a maximum of 22.42 °C just below the surface to a minimum of 22.19 °C at a depth of 7.12 m (Δ = 0.23 °C). The fluorescence characteristics suggested that chl-*a* biomass was dominated by an equal mixture of chromophytes (likely mostly diatoms and confirmed by microscopic observation, see Appendix A) and chlorophytes. The profiles for Eastern Pond (E5 in Table 1) showed temperatures that were higher than at the Binnaker and weak stratification (27.35 °C at surface, 26.83 °C at 1.05 m; Δ = 0.52 °C; Figure 2C) with fluorescence characteristics of the chl-*a* biomass dominated by chlorophytes (Fig. 2D). The abundance of indicator pigments for cryptophytes and cyanobacteria was very low at the Binnaker (<0.93 µg L^{-1}) and below the detection limit at Eastern Pond.

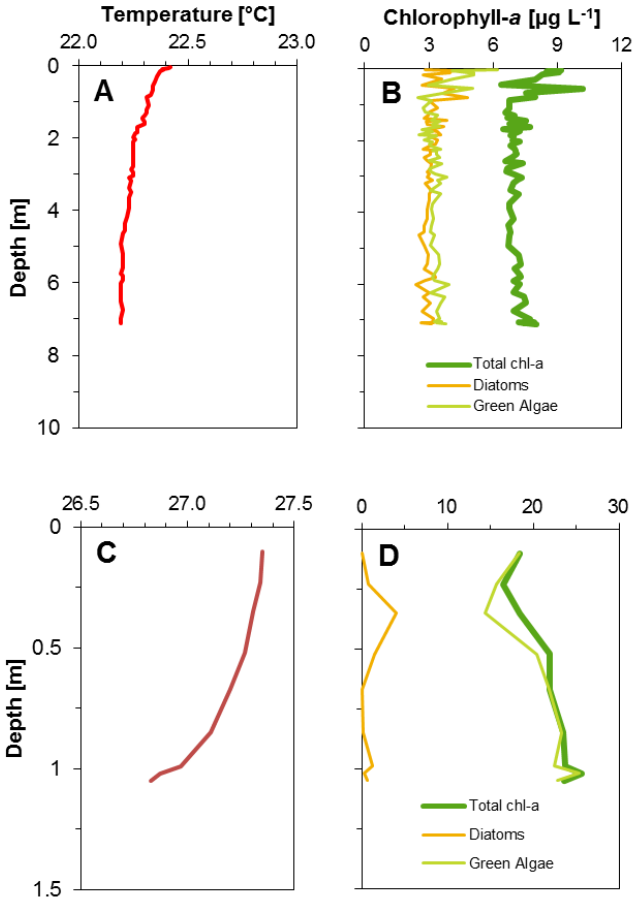


Figure 3. Vertical profiles on 20 July 2016 for temperature, chl-*a* and the taxon-specific pigments for diatoms and chlorophytes at the Binnaker (A, B) and in Eastern Pond (C, D). Note the difference in the scaling along the axes.

The mean chl-*a* concentration of 7.3 ± 0.68 µg L^{-1} at the Binnaker was typical of North Sea coastal areas in July (Alvarez-Fernandez and Riegman 2014). Chl-*a* biomass in Eastern Pond was higher (18.3 ± 1.11 µg L^{-1}) which may reflect the lower grazing pressure from the absence of herbivore grazers, the significantly higher water temperature that resulted in accelerated growth and possibly an increased input of inorganic nutrients from terrestrial run-off that stimulated phytoplankton biomass.

Oyster larvae: The abundance of oyster larvae at the Binnaker (Figure 2A) increased to concentrations above 1000 individuals per m^3 on three sampling dates (14 July, 28 July, 4 August) and suggested two main spawning events with maximum concentrations of 1868 individuals per m^3 (14 July) and 3737 individuals per m^3 (4 August). These concentrations were lower than values reported for the Bay of Marennes-Oleron in France (15,000-30,000 individuals per m^3, (Bernard et al. 2016)) but local conditions including the rates of mixing and its effect on dispersal rates and adult oyster density greatly affects these measurements so that direct comparisons with other locations are difficult. Furthermore, the timing of sampling in relation to a spawning event critically determines measured oyster abundance and sampling should have been conducted more frequently throughout July rather than increasing the sampling rate from the beginning of August only. No oyster larvae were found in Eastern Pond (Figure 2C).

C. gigas is oviparous (eggs are freely broadcast and fertilised externally) with development of the fertilised trochophore to the shell-bearing D-stage larvae taking about 2 (max 4) days depending on water temperature (Helm et al. 2004). Early D-stage larvae range in size from 70-75 µm length and our measurements for 14 July indicated lengths of 108 µm suggesting a fertilisation (spawning) date of 10 July (2 d development from fertilised trochophore to D-stage larvae and 2 days growth while in D-stage). Data for 4 August indicated an average shell length of the D-stage larvae of 86 µm suggesting a fertilisation date of 1 August (2 d development from fertilised trochophore to D-stage larvae and 1 day growth while in D-stage).

The 'biological zero' temperature (b_0) at which reproductive development (gametogenesis) starts is 8 to 12 °C in *C. gigas* and *O. edulis* (Helm et al. 2004). At adequate food supply, both species require 350 to 650 degree-days from the start of gametogenesis to the time they are ready to spawn (briefly, with a b_0 of 12 °C, five days at 20 °C would count as 40 degree-days: [20 – 12 °C] × 5d = 40 degree-days). Using a b_0 of 10 °C that was permanently exceeded from 30 April (mean daily water temperature of 10.1 °C) until the end of sampling, the gametogenesis for the first spawning event on 10 July took 72 days. Assuming an average water temperature of 17.5 °C for the period when no temperature data were collected at the Binnaker on 15 to 29 June (15 days), we calculated a value of 439 degree-days for gametogenesis from b_0 to the first spawning event. The 2nd spawning event on 1 August required 94 days from b_0 and amounted to 677 degree-days. Dating the onset of this spawning period a few days earlier to 25 July (87 days) when the onset of the 2nd spawning period was first noticed, resulted in 596 degree-days. These data are in general agreement with the predicted range of 350 to 650 degree-days from b_0 to spawning. However, logging of temperature data commenced on 23 April with a MDWT of 10.6 °C. This suggests that gametogenesis may have started before the selected date of 30 April when water temperature permanently increased beyond the 10 °C threshold. This could explain the earlier spawning event that resulted in high larvae abundance around 14 July and suggests that future efforts to improve spawning prediction should continuously monitor water temperature to increase predictability of the spawning date.

In case where similar calculations for *O. edulis* larvae were required, the brooding of larvae should be taken into consideration. This species is larviparous with a brooding period of 6-8 days resulting in larger larvae (170-190 µm length) upon release from the female that will take a shorter period of 10-12 days until settlement (Figure 1).

Summary and outlook

We used data collected at the Binnaker to describe the spawning behaviour of oysters in relationship to water temperature and the abundance of phytoplankton biomass (chl-*a*). The chl-*a* data suggested that food for the suspension-feeding oyster larvae was likely never limiting their growth and survival. However, further size-class determination of phytoplankton would be required to assess true availability of food to D-stage larvae at the correct size-class of 5-10 µm length (Helm et al. 2004).

The high-frequency temperature data suggested a close link with the timing of spawning. Increased release of oyster larvae coincided with maximum water temperatures around 24-25 July after an 11 day period of rapid increase in water temperature. This suggests that continuous measurements of water temperature may assist with an improved prediction of oyster spawning in the future. Collecting such information may become more important with the ongoing warming of surface seawater due to climate change and its effects on oyster biology including gametogenesis that could lead to annual spawning events earlier in the year.

Plankton identification and enumeration is labour intensive and future efforts may require alternative approaches to monitor spawning. Progress could be generated by the development of sensors that monitor gaping activity in adult oysters (non-invasive high-frequency valvometry (Bernard et al. 2016; Schwartzmann et al. 2011)). With additional funds from the Undergraduate Research Opportunities Programme (UROP) at the University of Essex, two students started developing a new sensor unit and a prototype is collecting gaping frequency data at the oyster rack in Pyefleet Creek since 14 July 2017. Further laboratory experiments including the temperature-induced spawning of oysters are planned for late summer and autumn this 2017. In the future, this sensor may provide the basis for an automated system that monitors and records spawning in oysters and reports this to COF and other interested parties.

Using the ponds to grow up oysters or produce viable offspring is currently hampered mostly be the insufficient supply of suitable food. Oyster larvae may be able to grow on the massively abundant small green flagellates, however, adult oysters would likely struggle to complete gametogenesis due to the insufficient supply of diverse phytoplankton (including diatoms) to produce fatty acids required for egg production.

References

Alvarez-Fernandez, S., and R. Riegman. 2014. Chlorophyll in North Sea coastal and offshore waters does not reflect long term trends of phytoplankton biomass. J. Sea Res. **91:** 35-44.

Bernard, I. and others 2016. In situ spawning in a marine broadcast spawner, the Pacific oyster *Crassostrea gigas*: Timing and environmental triggers. Limnol. Oceanogr. **61:** 635-647.

Helm, M. M., N. Bourne, and A. Lovatelli. 2004. Hatchery culture of bivalves. A practical manual, p. 177. FAO Fisheries

Nelson, T. C. 1928. Relation of spawning of the oyster to temperature. Ecology **9:** 145-154.

Ritchie, R. J. 2008. Universal chlorophyll equations for estimating chlorophylls a, b, c, and d and total chlorophylls in natural assemblages of photosynthetic organisms using acetone, methanol, or ethanol solvents. Photosynthetica **46:** 115-126.

Schwartzmann, C., G. Durrieu, M. Sow, P. Ciret, C. E. Lazareth, and J.-C. Massabuau. 2011. In situ giant clam growth rate behavior in relation to temperature: A one-year coupled study of high-frequency noninvasive valvometry and sclerochronology. Limnol. Oceanogr. **56:** 1940-1951.

Appendix A – Record of Sampling Reports (in reverse chronological order)

From: Steinke, Michael
Sent: 13 September 2016 15:11
To: 'Paul Harding (Paul@colchesteroysterfishery.com)'; "Graham Larkin' (Graham@colchesteroysterfishery.com)'
Cc: Randell, Luli S
Subject: RE: Oyster Larvae - latest sample (12 September 2016)

Dear Paul and Graham,

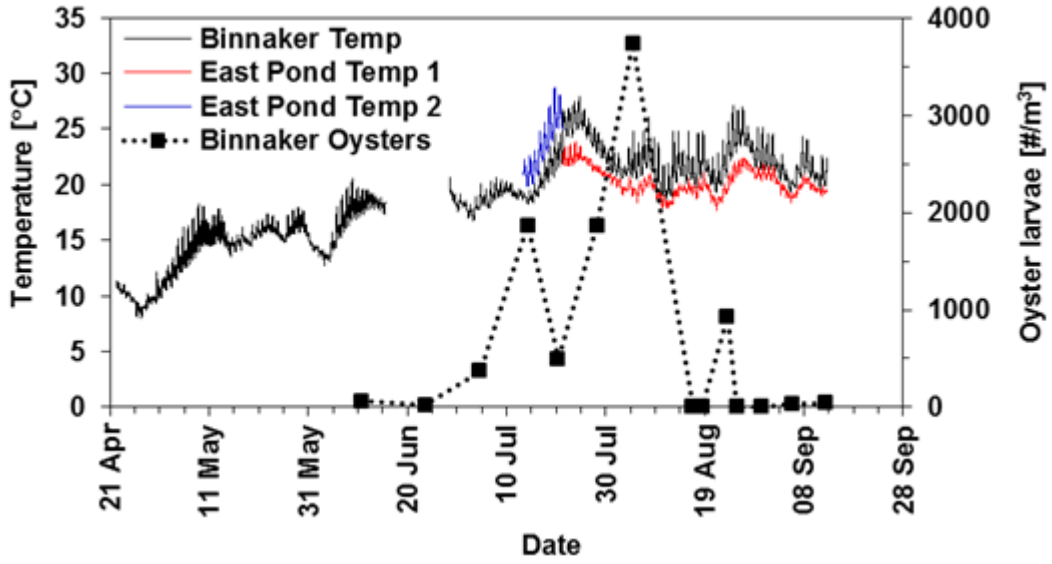
Alex provided plankton samples and delivered the temperature loggers on Monday (12 September 2016).

Binnaker
ZOOPLANKTON AND PHYTOPLANKTON: There is a shift to more dinoflagellates now. This is typical of this time of year, when relatively calm conditions and good supply of nitrogen (nitrates and ammonium) and phosphorous (phosphate) stimulate their growth. As a result there are more of the bioluminescent *Noctiluca* dinoflagellates (not a shellfish poisoning species) but also quite a few smaller dinoflagellates that **may** increase the shellfish toxins (I am not an expert in this area and it would need identification to species level or toxicological analyses to provide further info). However, larger diatoms are also noticeably coming back now which suggests that remineralisation of silicate from diatoms that died off over the summer and are buried in the mud is taking place. The general zooplankton is very similar to last week. Quite a few harpacticoid copepods (which is still surprising me) and a few *Littorina* snail larvae. Salinity of 35.

OYSTER: There are still very few D-stage and umbone-stage larvae around with a size of 90 to 180 µm which makes them approximately 4 to 9 days old. Numbers are very low with 44 larvae per cubic metre and they will settle over the next 9 to 14 days.
Last week I picked one oyster larvae and started to feed it in a petri dish. It is still alive but has not grown much (I kept it at 15 °C which might be too low). I have now isolated a few more larvae to (i) confirm that they grow into oyster spat (not cockles etc.), (ii) assess how difficult it is to successfully grow spat from that stage. Might be worthwhile inducing some spawning in *O. edulis* or *C. gigas* next season and grow them on in one of our tanks – if successful, we should be able to produce several 1000 spat?

East Pond
There is not much hope here – very little life. The phytoplankton community is dominated by small green flagellates that will not be good food for oysters (or indeed many other invertebrates). To run successfully, it would need some serious thinking and tinkering over the season. How feasible would it be to build ponds nearer to shore that are only inundated at high tide during spring tides? This way the water would be refreshed with new seawater every few weeks? Salinity is pretty high at 40 – I think oysters can handle this but it is not good for growth. Adjusting it by adding tap water may be an option but could also result in overcorrecting the salinity when the next rain will bring the salinity down further.

Data loggers
I have downloaded the temperature info from the data loggers (did you put in new loggers?). We have a good dataset for temperature now in order to correlate temperature with oyster spawning/larvae abundance data. The data is plotted in the figure below and you will see that there is a mismatch between two different data loggers from the East Pond (blue and red lines). I was hoping that the logger-to-logger accuracy is comparable – but it is not… I will have to calibrate all loggers in order to correct the temperature readings to the absolute temperature. I can do this once I have all four loggers back at base…
It appears that main oyster spawning appeared 4 to 8 days after a temperature peak of 26 to 28 °C on 20-24 July 2016.



Further sampling
We have collected 14 samples up to date and I do not see much reason in continuing any further since the numbers are pretty low and likely to stay low now. Originally, we had planned and costed 21 sampling trips. So, what would you like to do? Revisit the costings for this year and reduce the costs (less expensive for you) or pay up now for 21 sampling dates and use another 7 or so sampling trips next year (easier for us to administer)? We also have not managed to train up Alex sufficiently in using your or any other microscope for the identification of larvae. I wonder whether this is something we can still do now (next week?) or postpone until next year? Let me know what would you like to do, please.
I understand that your application for a voucher was approved by the University but I have not heard about the level of support from the University to finance this study. I will ask our admin team and pass on any further info as soon as I have it.

Future work
I am still very keen to put in an application to develop sensors for the monitoring of oyster spawning under this call: https://www.gov.uk/government/publications/agri-tech-catalyst-innovation-funding-in-developing-countries/competition-brief-for-agri-tech-catalyst-innovation-funding-in-developing-countries . I am in contact with one company that provided training and advice to pearl oyster farmers in Sudan in the past. It would be necessary to get them on board in order to be eligible for the above call. I am trying to establish contacts with oyster fisheries in Sierra Leone.

Being out on the water on Sunday was marvellous – many thanks again to you both, and especially Graham, for making this possible for my family and I.

Sorry for the long mail. Best, Michael

From: Steinke, Michael
Sent: 06 September 2016 15:44
To: 'Paul Harding (Paul@colchesteroysterfishery.com)'; "Graham Larkin' (Graham@colchesteroysterfishery.com)'
Cc: Randell, Luli S
Subject: Oyster Larvae - latest sample (5 September 2016)

Dear Paul and Graham,

Alex collected plankton samples on Monday (5 September).

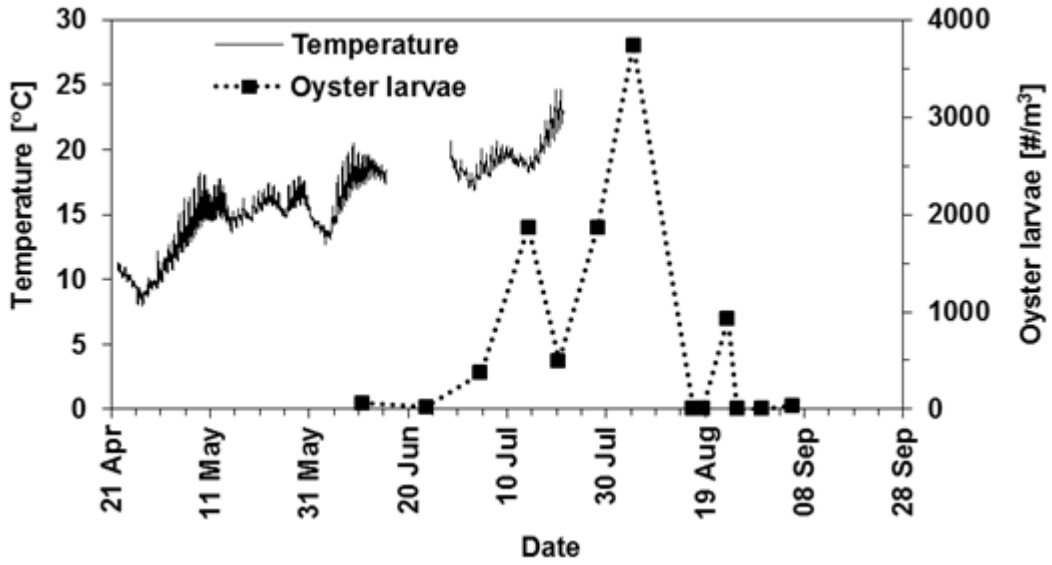
Binnaker
ZOOPLANKTON AND PHYTOPLANKTON: The general zooplankton and phytoplankton composition is very similar to last week. Quite a few calanoid and harpacticoid copepods still and a few more *Littorina* snail larvae again. The size of the diatoms is getting smaller and smaller – indicative of low nutrients, particularly silicate, that has been removed through the phytoplankton growth earlier. Salinity of 35.

OYSTER: There are still very few small D-stage larvae around with a size of about 110 µm which makes them about 4 days old. They will need approximately 2 weeks before they will settle. I have calculated an abundance of just 37 larvae per cubic metre of water, hence, they hardly register on the figure below.
I also saw another type of bivalve larvae that looked more like a *Mytilus edulis* (Common or Blue mussel). There are very few blue mussels at St. Osyth beach, so there is a small possibility to find them in the estuary. They may, however, also be *Mercenaria mercenaria* (Quahog or Hard clam) larvae – it's difficult to say.

East Pond
Same as last week. Alex noted a high number of insect/mosquito exuviae (the cast off the larvae leave when they hatch to the flying, adult stage). Surprised to find them at this high a salinity (salinity of 37, has come down a little from last week due to recent rainfall), but then the female mosquito cannot be too picky after the draught we had it is difficult for them to find suitable water for laying her eggs…

I will arrange another sampling with Alex for next week and hope that you can get the temperature data loggers replaced at a convenient time.

Best regards, Michael



From: Steinke, Michael
Sent: 31 August 2016 15:08
To: 'Paul Harding (Paul@colchesteroysterfishery.com)'; "Graham Larkin' (Graham@colchesteroysterfishery.com)'
Cc: Randell, Luli S
Subject: Oyster Larvae - latest sample (30 August 2016)

Dear Paul and Graham,

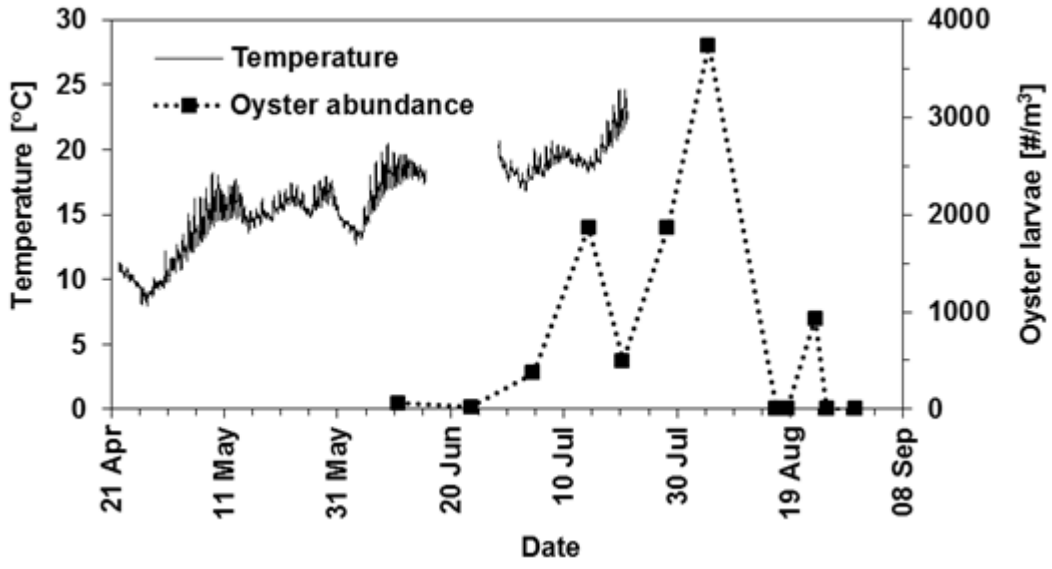
I went out with Alex to collect plankton samples form the Binnaker and East Pond yesterday (30 August 2016).

Binnaker
ZOOPLANKTON AND PHYTOPLANKTON: There is another clutch of calanoid copepod crustaceans going through. Numbers were also high for harpacticoid copepods that typically are associated with the surface and interstitial (space-in-between the grains) of the sediments. Not sure why so many in the plankton (water), may be due to disturbance upriver from cultching? Few polychaete and very few snail larvae now, and some *Noctiluca* dinoflagellates (but this is not a shellfish-poisoning dinoflagellate type). Many small centric diatoms.

OYSTER: None. I suspect that the oyster larvae all have come and gone now. I noticed your cultching activity and wonder whether (i) this may be not required (no larvae) and (ii) further cultching may actually do harm by smothering already settled larvae? I assume you do this anyway, but just to say that you should avoid laying new cultch onto the material that has been already out for the last 2-3 weeks…

East Pond
Very low diversity, many small flagellates, some ciliates including rotifers showing up. No copepods anymore (all dead?). Few nematode worms. No oyster larvae. Salinity relatively high at 38 (likely due to evaporation - Binakker was lower at 35).

Latest data:



I'd suggest that Alex takes another sample this Friday (just to be sure we are not missing any late spawning), and then we switch over to weekly sampling from next week.

Best regards, Michael

From: Steinke, Michael
Sent: 26 August 2016 16:24
To: Paul Harding (Paul@colchesteroysterfishery.com); 'Graham Larkin' (Graham@colchesteroysterfishery.com)
Cc: Randell, Luli S
Subject: RE: Oyster Larvae - latest sample (23 August 2016)

Dear Paul and Graham,

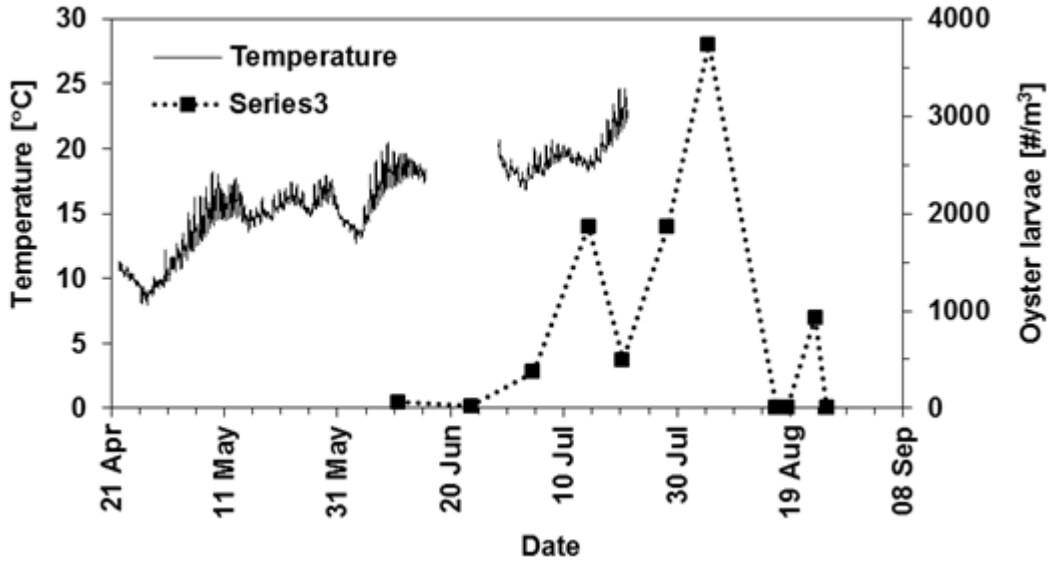
Alex provided samples this week on Tuesday (23 August) and Thursday (25 August). Luli is off sick and I do not have all the info for the latter sampling date yet. Here is info for 23 August:

Binnaker
ZOOPLANKTON AND PHYTOPLANKTON: Composition of the community very similar to last week.

OYSTER: small increase in numbers again, up to 934 larvae per cubic metre. Suspect that the recent temperature increase was driving another spawning event (however, from what I hear this was back down to 'zero' larvae on Thursday again… have to verify this with Luli next week). There might be a chance to capture a few more spat on freshly laid cultch towards the end of next week?

East Pond:
Low biomass, many flagellates, no oysters.

Latest data in figure format:



Alex has some 'fresh' temperature loggers and it would be great to exchange them with the ones in the field as soon as possible. He should then drop them off when he delivers plankton samples again next week (Tuesday?).

Have a good weekend. Best regards, Michael

From: Steinke, Michael
Sent: 22 August 2016 15:31
To: Paul Harding (Paul@colchesteroysterfishery.com); 'Graham Larkin' (Graham@colchesteroysterfishery.com)
Cc: Randell, Luli S
Subject: Re: Oyster Larvae - latest sample (18 August 2016)

Dear Paul and Graham,

Luli analysed the samples collected on Thursday 18 August. Here are the findings:

Binnaker
ZOOPLANKTON AND PHYTOPLANKTON: The plankton is shifting well into the summer communities with smaller plankton now more abundant - this is an indication of the decrease in inorganic nutrients (e.g., nitrate, phosphate) that have been taken up by the algae. Large diatoms are rare and zooplankton is dominated by polychaete (worms) and barnacle larvae. Numbers of snail larvae are much reduced and few late stages are in the process of settling.

OYSTER: No bivalve larvae found. Temperatures are expected to increase Tuesday and Wednesday - it will be interesting to see whether there is another spawning event by end of this week or early next week...

East Pond:
Very little biomass in general. Copepods have mostly disappeared, some crab and barnacle larvae. Many small phytoplankton (picoplankton - see below). No bivalve larvae.

I went through the calculations again and expressed the oyster larvae abundance as per cubic metre in the attached **Figure 1** (also showing the temperature data). I have noticed that there was some miscommunication between Luli and I so that some of the absolute numbers were actually higher - however, this did not affect the general pattern of oyster larvae abundance reported to you after each sampling. I am looking forward to receive the next batch of temperature data and then we should be able to make some predictions on the link between temperature and oyster larvae abundance. If this works, you may want to consider (next year?) to log water temperature data automatically so that this can be used as a predictive tool for larvae abundance. Alex may be able to check microscopically (with our help?) and inform your decision making.

I also analysed the data that we got from our Fluoroprobe instrument on 20 July 2016 (**Figures 1 and 2**). You will see that the estuary is well mixed (some stratification in the pond) and that the pond is dominated by green algae, likely the small picoplankton we have seen in the plankton samples in recent weeks and mentioned to you earlier. These green algae are not good food for oyster growth and egg production.

Best regards, Michael

From: Steinke, Michael
Sent: 18 August 2016 17:33
To: Paul Harding (Paul@colchesteroysterfishery.com); 'Graham Larkin' (Graham@colchesteroysterfishery.com)
Cc: Randell, Luli S
Subject: Oyster Larvae - latest sample (16 August 2016)

Dear Paul and Graham,

Alex sampled the plankton on Tuesday 16 August and Luli microscopically analysed the plankton composition and filtered water for biomass (chlorophyll-a) measurements.

Binnaker:
ZOOPLANKTON and PHYTOPLANKTON – The community has started to shift now. There is a high number of barnacle larvae present and still quite a few polychaetes (rag worm etc.). The number of snail larvae has dropped significantly. There are less diatoms and quite a few dinoflagellates showing up – this is typical for summer and could lead to shellfish poisoning problems due to the toxins produced in dinoflagellates.
OYSTER – Have not found any bivalve larvae at all. It has been 12 days since the last sample when we found relatively high number of larvae (about 0.6 per litre or 600 per cubic metre). These larvae were 80-100 µm in length which indicates an age of about 4 days at time of sampling. The development of the larvae takes 16-20 days (depending on temperature and food). So, with the warm weather (and plenty of chlorophyll-a biomass?), I am assuming that many of the first batch of oyster larvae will have settled now.
We will get more info when we read off the temperature data loggers next week (I will pass on 'fresh' loggers to Alex when he drops plankton samples next week – I think on Monday 22 August?) and we have quite a few frozen filters now to go into our biomass (chl-a) analysis soon.

East Pond
The community is much less diverse and many of the copepods have disappeared (insufficient food?). We found one barnacle cyprid larvae (the last planktonic stage before they'll settle down). The phytoplankton is dominated by tiny picophytoplankton (small flagellates) that readily clog our filters when filtering for biomass. Not good food for oysters I suspect…

I think Alex may have delivered another set of samples today (Thu 18 August) and Luli will look through them – more info soon…

Best regards, Michael

From: Randell, Luli S
Sent: 08 August 2016 11:48
To: Paul Harding; Graham@colchesteroysterfishery.com
Subject: Oyster Larvae - latest sample (4 August 2016)

On 8 Aug 2016 11:48, "Randell, Luli S" <lrandea@essex.ac.uk> wrote:
Dear Paul and Graham,

Alex sampled the Binakker and East Pond on Thursday 4th of August at around 16:00-17:00.

Binnaker:

ZOOPLANKTON and PHYTOPLANKTON – Starting to change in diversity since the last sample.
Littorina numbers have dropped, but those that have survived are much larger (grown more).
Phytoplankton seem to be less diverse. A couple of fish larvae could be seen.

OYSTER- Many more D-larvae compared to the last sample at 0.6 per litre compared to last week's 0.3.
Sizes vary from 80-101 µm in length. They were much more active (more were seen swimming in circles).

East Pond:

ZOOPLANKTON and PHYTOPLANKTON- Number of copepods have decreased in number, seemed to be an increase in *Daphnia* species.

OYSTER- In the east pond, there was one D-larvae when viewed under the microscope (approximately 61 µm), but none under the bioimaging microscope.
So they seem to at least be present.

Alex will be bringing the next batch at 08:30 this Thursday (11/08/2016).

Kind regards,
Luli

From: Randell, Luli S
Sent: 02 August 2016 12:19:48
To: Paul Harding; Graham@colchesteroysterfishery.com
Subject: Oyster Larvae - latest sample (28 July 2016)

Dear Paul And Graham,

Binakker and East Pond were sampled this week on Thursday (27 July 2016) [*NOTE: This should refer to sampling on 28 July 2017*] at about 07:00-08:00h by Alex.

NOTE: Due to miscommunication between the technician and I, samples were investigated a day later than they should of which meant the results will be less accurate.

Binnaker:
ZOOPLANKTON AND PHYTOPLANKTON –Similar to previous sample, but slightly less oyster larvae and more *littorina* species which have grown quite a bit now and can be seen to actively swim.
OYSTER – Found a couple of D-larvae approximately 124 µm in length, numbers are lower but as mentioned this could be due to the samples being left one day.

East Pond:
Same as the week before where there are copepods everywhere with smaller numbers of other zooplankton species.

The next samples will be investigated Thursday afternoon, but will update if any changes are made.

If you have any queries I am more than happy to discuss.

Kind regards,
Luli

From: Steinke, Michael
Sent: 21 July 2016 18:34
To: Paul Harding
Cc: 'Graham Larkin' (Graham@colchesteroysterfishery.com); Randell, Luli S
Subject: Oyster Larvae - latest sample (20 July 2016)

Dear Paul And Graham,

We sampled the Binakker and East Pond this week on Wednesday (20 July 2016) at about 13:00-14:00h.

Binnaker:
ZOOPLANKTON and PHYTOPLANKTON – Very similar to previous sample.

OYSTER – Found few D-larvae (straight hinge larvae) from 90-126 µm in length, so around four days old. The numbers are a little lower than last week - counted 0.5 per litre of water. We had some problems with the microscope that we use to take pictures with and for proper counts. Luli will give this sample another look tomorrow/next week and may have to correct the estimate above accordingly.

Nothing in the **East Pond** aside from lots of copepod (crustacean) nauplii larvae and copepodites to adults.

Temperature is now steadily increasing:

Maximum temperatures on the evenings of 19 and 20 July at 24.6 degC with average temperatures (+/- standard deviation) over the last five days of 21.7 +/- 1.34 degC.

I am off on holidays now till 12 August. Will be in touch once back in the office.

Best regards, Michael

From: Steinke, Michael
Sent: 17 July 2016 19:36
To: Paul Harding
Cc: 'Graham Larkin' (Graham@colchesteroysterfishery.com); Randell, Luli S
Subject: Re: Oyster Larvae - latest sample (14 July 2016)

Dear Paul and Graham,

Alex and I sampled the Binakker and East Pond on Thursday, 14th July.

Binnaker:
ZOOPLANKTON - Similar assemblage as on previous sampling day (4th July 2016). There is a second clutch of snail alrvae clearly on the way. Some small larvae in the plankton whereas the older, larger ones are setlling down and start crawling around.
PHYTOPLANKTON – Very similar to previous sample. Large diatoms dominated by (*Biddulphia* sp. and *Rhizosolenia* sp.). More smaller centric diatoms showing now.
OYSTER – Found a few D-larvae (straight hinge larvae) around 100-110 µm in length, so may be 5-6 days old. The numbers are increasing now - counted 1.9 per litre of water. Based on what I find in the literature (for example, see attached), this does not account for a proper spawning event. It should be about 5 per litre for a small spawning event and 15-30 per litre for a proper spawning.

East Pond:
Water is pretty clear with sediments settling out after the refill. The zooplankton is dominated by copepod crustaceans (hardly anything else...). Very little phytoplankton! This suggests that the copepods are massively blooming after the release from predator pressure and they are ingesting all the phytoplankton.

I am planning to sample again on Wednesday (20 July - from about 13:00h) before I am off on holiday (22 July - 12 August - I may miss the main spawning event...?). I have trained up my PhD student Luli Randell and will bring her on Wednesday to introduce to Alex. She will meet up with Alex when he brings samples to Uni while I am away.

I have looked at the attached scientific publication - they measure oyster gaping activity ('valvometry') remotely and can use this to infer spawning events - may be something to look into so that you can get the timings on your computer screen instead of taking samples and doing microscopy? Not sure how well this technique has been validated but worth a try? Happy to discuss further.

Best regards, Michael

From: Steinke, Michael
Sent: 04 July 2016 18:51
To: Paul Harding
Cc: 'Graham Larkin' (Graham@colchesteroysterfishery.com)
Subject: Oyster Larvae - latest sample (04 July 2016)

Dear Paul and Graham,

Alex kept looking at plankton all afternoon and left at about 6 pm tonight. Here are some results (please pass on to Alex):

Binnaker:
ZOOPLANKTON - Very diverse zooplankton community. The snail larvae (periwinkle = *Littorina* sp.) are noticeably less now and more copepod larvae (nauplii and copepodites) are now dominant. Also more polychaete larvae again.
PHYTOPLANKTON – Some large diatoms showing up (*Biddulphia* sp. and *Rhizosolenia* sp.). Still few *Noctiluca* sp. dinoflagellates present.
OYSTER – Found a few D-larvae (straight hinge larvae) all around 80-90 µm in length, so may be 4-5 days old. In total the numbers are still low (counted 0.5 to 0.6 per litre of water). Would not worry too much about putting out culch just yet – as you can imagine, I am expecting numbers to increase over the next two weeks (weather permitting).

Western Oyster Pond:
Generally the same as last sampling.
No oyster larvae, few copepods and many green flagellates.

Next sampling: Tides are a bit 'challenging' next week… May be Wed 13 Jul (HT = 7:25h) or Thu 14 Jul (HT = 8:20h) doable? I am relatively free on both dates. We could also try sampling late on Mon/Tue and keep the sample in fridge overnight (which is not quite as good as preparing the counts from fresh material). What would you recommend?

Best regards, Michael

From: Steinke, Michael
Sent: 24 June 2016 18:20
To: 'Paul Harding'
Cc: 'Graham Larkin' (Graham@colchesteroysterfishery.com)
Subject: Oyster Larvae - latest sample (23 June 2016)

Dear Paul and Graham,

Sampled for oyster larvae yesterday (23 June) at Binnaker and ponds.

Binnaker:
Generally a very similar story as on the previous sampling day (10 June).
ZOOPLANKTON - Very diverse zooplankton community, the snail larvae (periwinkle = *Littorina* sp.) are beginning to settle down but there are more younger, smaller snail larvae coming through, too. This time, the sample was dominated by barnacle larvae from very young nauplii to the later cyprid stage that will soon settle down.
PHYTOPLANKTON – Only few *Noctiluca* sp. dinoflagellates left now. Generally less diatoms which may reflect the enormous grazing pressure by the zooplankton.
OYSTER – Nothing to report here yet other than one living and one dead (empty) oyster larvae – so, reproduction has not kicked off yet!

Western Oyster Pond:
Generally the same as last sampling.
PHYTOPLANKTON - There are some large dinoflagellates in the sample. This may suggest that you could run into problems with the production of (shellfish poisoning) toxins later in the year. I suggest that you drain the ponds a.s.a.p. to refresh with fresh seawater. Look into the monitoring of nutrient levels in the ponds – they may be too high! I am happy to advise on this further if desired.
I also compared the salinities of both ponds with seawater. Came all out at 34 which suggests that the recent rain did not massively dilute the ponds and the different turbidity/colour does not stem from run-off of soil. I have no idea why the ponds look somewhat different!

I am planning to do another sampling on Monday 4 July. Would be good if Alex would be present and I will bring Russ our Technician who will soon take over the sampling. I will have to send you some more project cost info next week so that we can agree on a level of service/cost…

Best regards and have a good weekend, Michael

From: Steinke, Michael
Sent: 14 June 2016 13:52
To: 'Paul Harding'
Cc: 'Graham Larkin' (Graham@colchesteroysterfishery.com)
Subject: RE: Larval Testing

Dear Paul,

Sorry for the brevity earlier…

I had a look at the plankton samples on Friday afternoon. Here some info on the findings:

Binnaker:
ZOOPLANKTON - Very diverse zooplankton community, currently massively dominated by snail larvae (periwinkle = *Littorina* sp.) and crustacean (crab and copepod) larvae. Few copepodites and adult copepods.
PHYTOPLANKTON - Very diverse phytoplankton with various pennate diatom species dominating and good number of large dinoflagellates (*Noctiluca* sp.).
OYSTER - Also found 1 (one) bivalve larvae that could have been from oyster. This gives a THEORETICAL abundance of 0.1 oyster larvae per litre of water – not many!

Western Oyster Pond:
ZOOPLANKTON - Very low diversity with few crab larvae and harpacticoid copepods.
PHYTOPLANKTON – massively abundant green flagellates.
OYSTER - No oyster larvae.

I also took samples for storage in Lugol's iodine (could be used later for more detailed analysis if required) and filtered water for the estimation of plant (algal) biomass using chlorophyll quantification. These are in the freezer and will be analysed once we collected a decent number of samples.

You have asked about advice on the emptying and refilling of the oyster ponds. Based on my microscopy, I would rate the plankton composition in the pond as poor food for oysters (green flagellates will be eaten but are generally poor in essential fatty acids for egg production). You may rectify this shortcoming by emptying the ponds now, check for spat from last year and refill with water from the estuary (can you pump this up?). The risk of introducing *Crassostrea gigas* (rock oyster) with the refill water is low (only few larvae present in river) but you may have an explosion of periwinkles in the ponds (however, some of the larvae will be filtered out by the oysters quickly). However, you will also introduce the nutritionally more appropriate diatoms that will hopefully grow and provide a steady supply of food.

If you want to invest, you should look into weekly testing of ammonium/ammonia nitrate levels and pH in the ponds using aquarium kits to help with decision making in the future. Also, may be consider growing diatoms in bags to supplement the ponds?

I will ask the students to take a fresh temperature logger with them so that you can put this in place for the one you will retrieve. Take note of the dates of retrieval and launch of these loggers, please! You already purchased the plankton net (you will be invoiced for this at some point) but I wonder whether you are ready to purchase a few of the temperature loggers so that we have more flexibility? Would be good to have at least one in the ponds, too! I quoted for the loggers earlier: £59.99 each – you could do with 6 of those. Let me know and I could order from here.

Let me know if you need more interpretation of the points above. Happy to chat on phone.

Best, Michael