Digitised notebook for MERP minicruise PQ4/15, 30 April 2015, to L4, Plymouth.

*Do you have anything to add Angus?*

Departed Sutton Harbour, Plymouth at 16:40 on 30th April 2015 after waiting for several days due to poor weather.

On arrival at the L4 buoy we observed a scallop dredger with it's dredges caught on part of the L4 mooring (and took photos), although she later managed to free herself.

Gear deployed:
Jelly net – a 1 $m^2$ 500µm mesh square frame net towed in a double-oblique profile from the surface to seabed and back again. Each net takes about 20 minutes and covers around 1km.
Ring nets - WP2 rings with 200µm and 63µm meshes, deployed in a single vertical haul, side by side.

Labelling of samples:
Most samples were labelled with the following code: Cruise (PQ 4/15), Day or Night (D, N), Net (J or Jel, 200, 63) and the replicate (1-4). A further description of the sample preservation method or species preserved was included. Any samples labelled without day or night will have been collected in order (e.g. Jelly net 1-4 by day; 5-8 by night).

Deployments:

Jelly net 1 (PQ4/15, D J 1), shot at 17:37, on board at 18:00. The sample in the non-filtering cod end (5 litres) was roughly divided with half frozen and half kept for picking out of animals. The frozen half was filtered on a 200µm mesh, which was then put into a freezer bag with the sample and frozen. It remains to be seen if this contaminates the sample, but was the most practical method with large volumes of gelatinous material. See notes.
Large numbers of Pleurobrachia were observed (but not counted) with a varying range of sizes. Twenty-five individuals were measured to get a size range (6-14mm long) and ten individuals snap frozen in liquid Nitrogen to preserve their gut contents and DNA. Samples were then put into a -20°C freezer until they could be relocated to -80°C in the lab. There were a range of different, unidentified, fish larvae in the sample and a number of these were frozen together in a bag.

Jelly net 2 (PQ4/15 DJ2), out at 18:30. Half the sample frozen in a bag as it was too big for a falcon tube. Half the sample was put into 4% Formaldehyde (Formalin) and preserved.

Jelly net 3 (out at 18:51). Half frozen, half kept for picking specimens.
Ten Pleurobrachia ? pileus were snap frozen from this sample, along with one bag of fish larvae and two Hydrozoans which were probably Leukartiara sp. Another 25 Pleurobrachia were measured (6-12mm), although it is probable that smaller individuals were present but not visible to the naked eye in the dim lighting conditions.

Jelly net 4 (Out at 19:10). Half frozen, half into ethanol. There were definitely more Pleurobrachia in the sample and I observed a much more fragile and larger ctenophore with greyish ctene-rows. My best guess would be Bollinopsis. This had not been spotted in previous samples on this station.

Ring net sampling (19:15).
Ring net pair 1 – 200um (PQ4/15, D 200-1) and 63um (PQ4/15, D 63-1). Half was frozen and half into Formalin. We ran out of spare 100um mesh at this point and so frozen samples after this time were semi-quantitative and probably only 95% of half the sample.

Ring net pair 2 – PQ4/15, D 200-2 and D 63-2
Half frozen and half preserved in ethanol.

Ring net 3 - PQ4/15, D 200-3 and D 63-3
Half frozen and half discarded as nothing was picked out. For each of these nets the 200um was semi-quantitative and added to freezer bags, while the 63um was carefully sieved and preserved in a single falcon tube.

Ring net 4 – Same as Ring net 3, half frozen, half discarded. Bag for 200um and Falcon for 63um.

Night samples.
We waited until dark having completed the day samples and shot the net at 21:20. Dolphins were swimming around the ship at dusk and rather enjoying themselves! There were an estimated 20 individual Common Dolphins around Plymouth Quest, often languidly resting at the surface as well as doing their usual wild antics.

Jelly net 1 (PQ4/15, N J1), out at around 21:40.
Half this net was frozen as per normal and the other half kept for picking organisms. In this net were another 7 of the Leukartiara (?) hydrozoans (labelled PQ4/15 NJ1 Hydro 1-7) measuring 8-12 mm long. These were all picked out and frozen for molecular analysis. Approximately 30 fish larvae were also frozen in a bag collectively and another 10 Pleurobrachia ?pileus were frozen. The Pleurobrachia measured between 7 and 14mm in the 25 measured individuals.

Night Jelly net 2. Half this net was frozen and half preserved in Formalin. There seemed to be many crustacean (? Decapods) larvae in this and the other samples at night as the sieve was vibrating with all the small movement.

Night ring net 1 (PQ4/15, N 200-1 and 63-1). Net out at 22:10
Half was frozen and half put into Formalin

Ring net 2 (PQ4/15, N 200-2 and 63-2). Net out at 22:15.
The 63um cod end was lost and this sample was collected (and half was frozen) after the other ring nets had been completed. The 200um net was half frozen and half went into ethanol.

Night jelly net 3 (PQ4/15, N J3). Half this net was frozen and half had organisms picked from it. We froze 10 Pleurobrachia ?pileus, 6 ?Leukartiara (labelled hydro 1-6), 1 Obelia or Cosmetira (fragile circular jelly 35mm diameter with a cross in the centre and black dots around the edges), 1 large larval fish and a bag of around 40 larval fish. The size Leukartiara (?) were measured between 12 and 19mm high, and 25 P. pileus (?) between 6-13mm long.

Night jelly net 4. Half frozen, half into ethanol.

Night ring net 3 (PQ4/15, N 200-3 and N 63-3). On deck at 23:10. Half the sample was frozen and half discarded (there was one fish larva in the 200um and no obvious ctenophores in either net).

Night ring nets 4. PQ 4/15, N200-4 and N 63-4). The 200um net was half frozen and half discarded. The 63m net was half frozen and half into ethanol to make up for the missing N63-2.

Night ring net 5. This was labelled at PQ4/15 N 63-2 to make up for the missing cod end on Night ring 2 and preserved as half frozen, half discarded.

Sampling ended at 23:30 and we returned to Sutton Harbour by 00:30.
Crew – Andy Perkins, Jim, Al? and an engineer.
Station positions are in the file 'MERP PQ4-15 Positions 3-4-15.xlsx' and unformatted in 'MERP 30-4-15.txt'.

Notes:
Bottles of ethanol and for Formalin do not need to be 1 litre in size for the single-dip ring nets. Half litre or smaller would be sufficient. They do need to be this big for the Jelly-net samples though.

One 63um cod end lost.

Alternating pairs of ring nets and pairs of jelly nets stops the work becoming too intense during the ring netting, but it does mean keeping more of an awareness of which samples have been preserved in ethanol and formalin already. Picking on the first of two jelly nets gives a 20 minute window to pick, measure and freeze animals. Doing this with a ring net following fast on the jelly net's heels is just not practical.

Q - Do sieves contaminate the molecular samples or can we also preserve the sieve mesh? If we can then Angus would prefer to enlarge the sieve to allow for quicker sieving. Ideally we would also take aboard large quantities of 100 (?) um mesh for cutting into sieve material.

There is a need for two pairs of 1.5-2 litre beakers for splitting the samples. At least five buckets are also needed, and more curved forceps. A second 53um sieve would also be useful and two 100 or 200um sieves essential for speedy sorting of the samples.

Q - How important is the $4^{th}$ sample? It is just in case of mistakes? Statistically the seasonal signal is going to be more variable than the replicates within a time-point and so it feel like we are filtering and sieving more than is required. If there was a bloom or massive jelly catches it would be a nightmare. This trip was okay.

Measuring fragile jellies on board is possible, but not easy. The firmer species are fine. The size means there is probably a bias towards the larger organisms and it would be worth going through the Formalin samples to get a real size range. The more transparent the species, the more bias towards the larger individuals, however I was using a tea strainer to pick up multiple Pleurobrachia together which should have randomised the selection process a bit. Measuring biomass or displacement volume of these individuals would be much more difficult and would have to be done by measuring the volume of several (10?) individuals of similar size at the same time. This was not tried on this trip. They would have to be added to a known volume of water and the water change

noted. Mass-size ratios are otherwise required. Is there a known change in mass on formalin preserved samples?

Leukartiara ?sp (?octona).
Identification was difficult in the light and this needs confirmation. These jellies are much softer than Pleurobrachia and it is unknown if we will be able to extract the stomach contents from them. Tentacle bases were well separated from the organ (and stomach?) mass higher up the bell.

Fish larvae
Q - Can fish larvae be separated for molecular work afterwards or do they have to be frozen separately? There was plenty of diversity, but rarely more than 3 or 4 individuals of most species. There is potential for future work here, but it may be that the samples of mixed individuals are not workable; and the fish may have voided their guts as the net came in. They were all swimming happily though in the white trays so we may be okay.