# Methods

## Study site

A map of the river

Description automatically generatedThe study site comprises a system of saline lagoons that are part of the Llanelli Wildfowl and Wetlands Trust (WWT) Centre at 51.6650° N, 4.1252° W. A conservation area designated for the protection of habitats that support bird diversity within the Burry inlet on the Loughor estuary, South Wales, UK (Figure 10.1). Saline lagoons where the sampling was conducted were artificially created from salt marsh habitat in 1998 to improve fish stocks in the area and attract coastal and migratory bird populations. These lagoons are classified as isolated as they are separated from the sea for the majority of the month. Sea water only enters the lagoons by overtopping during high spring tide (> 8.2 m) or ground water seepage, they also typically have variable salinity that is often low (Downie, 1996; Joint Nature Conservation Committee, 2019). The lagoons are surrounded by saltmarsh habitat and to the north is a 2km sea wall, which prevents any landward migration of marsh vegetation.

***Figure 10.1 Map of Llanelli WWT and surrounding area***

Our study site, the Llanelli Wildfowl and Wetland Trust (WWT) is located in Llanelli, on the Loughor estuary, South Wales, UK (51.6650° N, 4.1252°W).The wetlands here are part of the Burry Inlet Site of Special Scientific Interest, indicated by the spotted line at the sea-land connection of the Loughor estuary.

A map of a bird

Description automatically generatedThe three lagoons studied have a combined area of 45,600m2. Two larger lagoons: the lookout side lagoon and the sea-side lagoon (LSL and SSL respectively), are both approximately 20,000m2 and have a drainage point, they are also connected by a small gap in the barrier between them that formed due to erosion. The smaller lagoon is approximately 5,000m2 but was not sampled due to excessive debris (Figure 10.2). Depth varies greatly predominantly due to tidal variation, at the highest spring tides of 9.5m the lagoons have an approximate depth of 1.5m and all barriers separating the lagoons are also submerged. When the tide recedes, water drains out over the top of the lagoons or via the drainage points in the two larger lagoons leaving between 10-40cm of water depending on the time of year. The drainage points are controlled by staff at the wetland centre allowing control over the amount of water to be drained.

***Figure 10.2 Sample site and characteristics of Llanelli saline lagoons***

*There are three saline lagoons, the lookout side lagoon (LSL), the seaside lagoon (SSL) and the small lagoon (SL). LSL and SSL are connected due to a break in the wall separating them. SL is isolated except during high spring tides, this however was not sampled due to debris that would rip the net. The man-made characteristics of the site include the lagoons systems, sea wall, bird look out, drainage points and wire fence. The natural characteristics are the surrounding marsh area, and tributaries, marsh grasses, reeds and samphire that grows in the lagoon. Seine deployment sites are shown in the top centre of LSL, and top right corner of SSL. Seines were deployed perpendicular to the shore from a bank that was sloped and covered an approximate area of 25m.*

A screen shot of a graph

Description automatically generatedThe tidal cycle occurs monthly with two neap tides and two spring tides, one spring tide has a greater high tide than the other and it is during this stage of the month that the saline lagoons usually flood (JNCC, 2019; US Department of Commerce, 2012). From a community ecology perspective this flooding has been treated as a perturbation on the community as it disturbs the water causing mixing and increasing salinity as well as potentially introducing predators to the habitat (Alcérreca-Huerta et al., 2019; Hairston et al., 1960). The tidal cycle for the sampling period (February to July) is shown in Figure 10.3 where lagoonal flooding only occurs when the tide height is greater than 8.1m. During the time the lagoons are not flooding, the community could be considered to be “recovering”. Conversely, this could act the opposite way round, especially during summer when water temperature rapidly increase during the “recovery” phase and the water becomes stratified producing an anoxic bottom layer (Tyler et al., 2009).

**Figure 10.3 Llanelli high tide dates and heights**

*Llanelli high tide heights for entire sampling duration (February to August), each peak represents a spring tide and each trough a neap tide. The horizontal line symbolises the tide hight that needs to be exceeded for the lagoons to flood. June is highlighted where the tide hight does not exceed the flooding height (POLTIPS, 2022).*

## Sampling methodology

Aquatic communities comprising fish, malocrustaceans and bivalves, polychaetes, insects and plant species (see Table 10.1 for species list) was sampled monthly between February and July 2024 (excluding June as the lagoons did not flood, see Figure 10.3. The two larger lagoons, LSL and SSL, were sampled four times per month: approximately 24 hours before flooding; the halfway point during flooding (this varied depending on the number of days the lagoons flooded); approximately 24 hours after the final flooding; and approximately 48 hours after that during the recovery phase (named BF, DF, AF and REC respectively) shown in Figure 10.4. This sampling design allowed us to determine the impact of a suspected environmental perturbation (flooding) on the community and recovery timeframe, if recovery occurs at all. The community was sampled using a seine net, to ensure all species that inhabit both pelagic and benthic environments were surveyed (Franco et al., 2022).

### Seine

The sample design consisted of 120 sampling events (2 lagoons x 5 months x 4 days x 3 replicates). Seine nets were chosen as the preferred catching method based on their potential to maximise catch in saltmarsh habitats (Franco et al., 2012, 2019; Verdiell-Cubedo et al., 2013). The seine net used was 15m x 2m with 3mm mesh, attached to 1.5m poles, an effective net for targeting smaller benthic-demersal species (predominantly between 20–100mm) (Franco et al., 2022, 2012). For each pond all three replicates were taken from the same location throughout the study (shown in Figure 10.2). This was to increase replicability as different microhabitats within the lagoon may have varied communities and also because there were a limited number of places the seine net could be deployed as the lagoon banks were too steep at most points. Seine hauls were completed by two people, deployed perpendicular to the shore, each covering an area of approximately 20m2.

Once a seine has been completed fish and invertebrates (excluding crabs which were placed in a separate bucket) were transferred from the net to a filled 40l bucket to be processed. After all specimens had been placed in the bucket, individuals of each species were counted using a hand net then placed into a second filled bucket. Fifteen individual fish of each species were measured to the nearest 1.0mm using a WaterMark Ultimate Fish Board. Seines one and two for each lagoon remained in covered buckets until the third seine was completed to avoid recatch. After processing the catch was released in the same location it was captured.

***Table 10.1 Taxonomic classifications of species observed at Llanelli saline lagoons***

Taxonomic classification of species recorded during sampling. The first column shows the name used during field work, where species were given the same recorded name multiple species were observed but due to high volume recorded under the same category. Species were recorded to the highest taxonomic level possible (Class, order and species). Species without a recorded name were observed but their abundance was not recorded, and they were excluded from the analysis (Cramp and Simmons, 1977; Franco et al., 2022).

Recorded name Class Order Species Common name

Teleostei

Gobiiformes

Goby spp. *Pomatoschistus microps* Common goby

Goby spp. *Pomatoschistus minutus* Sand goby

Mugiliformes

Mullet spp. *Chelon spp.*  Grey mullet

TH mullet *Chelon ramada* Thin lipped grey mullet

Gold mullet *Chelon auratus* Golden grey mullet

Flatfish Pleuronectiformes Flatfish

Flounder *Platichthys flesus* European flounder

Sole *Buglossidium luteum* Solenette

Anguiliformes

E. eel *Anguilla anguilla* European eel

Malocostraca

Amphipod Amphipoda Amphipod spp.

Mud scud *Corophium volutator* European mud scud

Decapoda

Shrimp spp. *Palaemon varians*  Atlantic ditch shrimp

Shrimp spp. *Palaemon elegans*  Rockpool shrimp

Shrimp spp. *Crangon fabricius*Brown shrimp

EG crab *Carcinus maenas* European green crab

Bivalvia

Cardiida

Cockle spp. *Cerastoderma edule* Common cockle

Polychaeta

Spionida

Polychaete *Polydora spp.*  Bristle worms

Insecta

Diptera

Blood worms *Chironomid spp.* Chironomidae midges

Larvae *Tipula spp.* Cranefly larvae

Coleoptera

*Gyrinidae spp.* Whirligig beetles

Liliopsida

Cyperales

*Spartina spp.* Cordgrass spp.

Ulvophyceae

Ulvales

*Ulva lactuca* Sea lettuce

Magnoliopsida

Apiale

*Crithmum* *maritimum* Rock samphire

A graph with lines and dots

Description automatically generated**Figure 10.4 Example of a sampling schedule for the month of February**

*Sample schedule for February based on high tide heights and approximate flooding period of six days. The graphs shows the different sample dates: 09/02 ~ before flooding, 12/02 ~ during flooding, 15/02 ~ after flooding and 17/02 ~ recovery (POLTIPS, 2022).*

### Water Assessment

Water measurements were taken during the sampling period, at the same locations where seine nets were deployed, prior to seine hauls to avoid water mixing. A total of 80 measurements were taken (2 ponds x 5 months x 4 days x (1 measurement + 1 water sample)). Temperature and dissolved oxygen (DO) were measured in the field using a DO probe (consistently submerged by 10 cm during all measurements). Water samples were collected from each lagoon to measure chlorophyll concentration in the lab. For this, 100 ml of water was collected and added to a container already containing 300 ml of 99% ethanol, resulting thus in a 75% ethanol solution that allowed us to euthanise zooplankton and prevent change in chlorophyll concentration (Black and Dodson, 2003; Joyce et al., 2005). Water samples were then tested in the lab using a multiparameter sonde (insitu device) to measure pH, salinity, turbidity, and chlorophyl-a Fluorescence. Two measurements from each sample were taken and an average calculated. Since three quarters of the water samples was almost pure ethanol, obtained measurements were multiplied by four to approximate the true values.