

Overwintering Oysters

Although the majority of the literature recommends overwintering oysters in bags or in other structures that have been sunk to the bay bottom, there are some production areas where this is not possible. Very little research has been conducted on alternative storage arrangements in the American Oyster (*Crassostrea virginica*). Morse (2006) ultimately recommends overwintering oysters in a separate bay if the grow out bay is not suitable for winter storage. Seaman & Schwarz (1987) only recommend overwintering oysters on land if oysters can be stocked at a high density, if water quality can be maintained at a high level, and if energy costs are minimized.

The sections below contain information on alternative holding systems and conditions that impact the survivability of American Oysters through winter storage. It should be noted that Kennedy et al (1996) caution against extrapolating scientific studies from one geographic region to another, since the American Oyster is found over such an extensive range and genetic variations have been noted between populations across its range. Thus populations of oysters from different geographic ranges may react completely different to storage situations than oysters from studied populations. It is also recommended that testing new storage methods occur as small scale studies before moving to commercial storage levels (Morse 2006).

1.1. Alternatives to on bottom overwintering of American Oysters

1.1.1 Dry Storage- Air

1.1.1.1 Study A (Hidu et al 1988)

Hidu et al (1988) studied the storage of oysters in humid air conditions at multiple treatment temperature (0°C, 3°C and 6°C) for a maximum of 6 months. In this study, oysters of three different size classes (7-9mm, 12mm and 40mm) were removed from air storage at monthly intervals from each of the temperature treatments and placed in ambient sea water tanks with cultured algal mixtures post storage. One month after the longest storage treatment (6 months), oysters were sorted as either dead, live with new shell growth, or live with no new shell growth. To further evaluate the viability and strength of the oysters in both living groups, a subsample was maintained for an additional month at warmer water temperatures and with algal cultures. Oysters from this additional trial either died, or put on new shell growth and survived. This experiment by Hidu et al (1988) found that oyster size had an impact on survival rates but storage temperature did not. Survival rates also varied based on the duration of humid air storage (1-6 months). The survival rate of seed oysters was over 80% for all storage treatments in months 1-5; however, survival rates dropped in the oysters that were maintained in humid air storage for 6 months. Oysters that were in the 40mm size class had a much higher

survivability (95%) across temperature treatments then either of the smaller oyster groups up to the 6 month treatment time. The 12mm oysters had lower survival rates than the 40mm oysters; however survivability in this size class was still quite high >85%. Humid air storage did not impact subsequent survival rates in the following growing season. Survivability in oysters from the 40mm size class of the humid air storage treatments was at 95% after the first summer post treatment. Shell length was not impacted by storage length although oyster weight did decrease in oysters that were stored for the longest treatments (6 months). This is due to the fact that some of the longer treatments lasted into the growing season giving these oysters less time in optimal growing conditions.

In some of the longer storage treatment groups, oysters appeared to be dead (desiccated tissue, no response to probing and no evidence of a heartbeat); however, when these oysters were incubated in warm water with algal cultures they began to feed and showed a survival rate of 80% (Hidu et al 1988).

Due to the positive results of the initial (Hidu et al 1998) study, an oyster farm in Maine overwintered 120 000 oysters from December- May in humid air storage with an overall survival rate of 90% (Hidu et al 1988). Oysters in this trial were held in 1" mesh bags that were covered with moist burlap and seaweed. Air temperatures ranged from 1° C- 10° C throughout the duration of the study.

It is important to note that both of the Hidu et al (1988) experiments were conducted on populations of oysters in Maine, and therefore survivability may differ in other regions due to genetic variability in oyster stock, variability in physiological condition of oysters prior to storage, variability in storage conditions and variability in disease statuses in different populations of oysters from different geographic areas.

1.1.1.2. Study B (Wang & Amiro 1977)

Wang & Amiro (1977) studied the impact of humid air storage on American Oysters grown in Prince Edward Island, Canada under different conditions using condition index, coliform counts, chemical analysis, taste panel assessment, percent free liquor and weight loss, valve activity and survivorship as indicators of oyster quality post storage. Oysters in this study were maintained in air storage for a maximum of 6 months to evaluate monthly changes in oyster quality. Oysters were held in cold rooms at temperatures maintained at 2-5° C under a variety of humidity levels and holding conditions. Oysters in the first treatment group were placed in 6 millimeter mesh bags at a stocking density of 40 oysters/bag. The entire unit of bags in this treatment group was then wrapped in a 6 millimeter wrap of polyethylene in a tent like manner. In this first treatment group, humidity was set so that the internal surface of the wrap surrounding the mesh bags was continuously saturated with water vapour. The second humid air treatment group involved oysters packaged as described above in mesh bags, but without a tent-like wrapping leaving oysters exposed to humidity levels ranging from 55-70%. In this

treatment group oysters were arranged in the bag so that every oyster received the same level of air exposure. The third treatment group in this study involved oysters that were packed in polyethylene bags, however these bags were stored in an upright position causing oysters to fall on top of one another in a dense pile (as opposed to the horizontal placement in treatment 2). In addition, the oysters held in treatment three were held in bags that were twist tied right to the surface of the oysters, minimizing the amount of air loss from the bag and exposing oysters to humidity levels generated by the oyster metabolic processes themselves. In treatment four, oysters were placed in 5 gallon plastic tubs instead of polyethylene bags at a density of 240 oysters/tub. All tubs were securely sealed with lids allowing the oysters to be exposed to humidity levels generated by their own metabolism. Of each of the holding methods tested in this study, storing oysters in bulk quantities in air tight polyethylene bags and plastic tubs had the highest level of success in the dry overwintering storage of oysters (Wang et al 1977). Quality in all air held treatment groups maintained a high level until 3 months, after which quality deteriorated monthly to the 6 month end point of the study. In all four treatments mentioned above 50% mortality levels were reached between 3.5-5.5 months depending on treatment type. Oysters in the dry air storage groups utilized lipid and carbohydrate reserves throughout the duration of the holding study. Of all oyster grades used in this study, choice oysters had the best outcomes after humid air storage treatments (Wang & Amiro 1977). Overall survival by the end of the 6 month holding experiment was 60% for choice oysters and 48% for standard oysters. Freeman & Lavioue (2003) found similar trends between the survival of choice and standard oysters in dry storage conditions. It is believed that choice oysters withstand dry storage better than standard oysters since their cupped shape allows them to retain fluid better when gaping occurs (Freeman & Lavioue 2003). Taste tests indicated that oysters stored in air storage became unpalatable by four months. Medcof (1958) also found a decline in palatability in air stored oysters as compared to wet stored oysters, which he attributed to the buildup of metabolic waste products in air stored oysters as compared to wet stored ones that were able to flush metabolic wastes out of their tissues and into the water column. Faecal coliform levels in oysters stored for 6 months in winter storage did not change much over the duration of the overwintering storage project. Wang & Amiro (1977) attribute this to the fact that coliform bacteria generally do not multiply efficiently at low water temperatures.

1.1.2 Dry Storage-Refrigerated

Refrigerated rooms and commercial coolers have also been used to overwinter American Oysters. It is recommended that oysters held in these conditions remain at stable temperatures between 0°C-3°C under moist burlap cloths that maintain humidity in the refrigeration unit yet do not allow oysters to become submerged in standing water (Morse 2006). In refrigerated systems oysters should be periodically checked for mould in case humidity levels are too high within the storage unit. In addition, oysters that are stored in dry storage systems should be placed with their cupped valve down, to ensure that the liquor remains inside the oyster, delaying desiccation (Morse 2006)

1.1.3 Dry Storage-Buried

Oysters can also be stored in underground pits during the overwintering period. Pit storage systems must be in areas with excellent drainage, be below the frost line and be insulated to moderate temperature fluctuations throughout the winter season (Morse 2006). Oysters in a pit system should be layered in burlap during storage (Morse 2006). Oysters have also been stored for short terms by being buried in seaweed in cold rooms throughout Cape Breton, Nova Scotia (Freeman & Lavoie 2003).

1.2 On land wet storage

Wang & Amiro (1977) also tested wet storage as a method to overwinter American Oysters in Prince Edward Island. In this study, oysters were maintained in tanks of filtered sea water at a temperature of 1-2°C. Oysters maintained for 6 months in wet storage had significantly higher survival rates than the four methods of air storage that Wang & Amiro (1977) examined in their study. Wang & Amiro (1977) also found that oysters stored in tanks of water scored better in taste tests than air stored oysters.

Seaman & Schwarz (1987) also conducted an experiment to evaluate wet storage as an overwintering holding strategy for Pacific Oysters (*Crassostrea gigas*) in static tanks without the addition of feed. In this experiment, *C. gigas* was stored at high densities (400 kg/m⁻³) in tanks of water supplied by a salt water well that maintained a water temperature of 10.5°C. Aeration was provided to tanks in this experiment by pumping water through 7 m lengths of porous PVC pipes. Water changes were conducted three times/week from November-January and two-three times a week from February-April. In this experiment, mortality was negligible and oysters maintained a high level of quality and a high condition index through to the end of the 6 month holding experiment. The use of a salt water well in this study reduced energy costs and made the storage feasible for the commercial partner involved in the study (Seaman & Schwarz 1987). Ammonia and nitrate levels were also monitored on a weekly basis and although oysters were metabolically releasing ammonia as waste, bacteria present in the oysters and in the tanks brought about the spontaneous nitrification process which allowed water quality to remain high throughout the duration of the experiment.

Ultimately Wang & Amiro (1977) and Freeman & Lavoie (2003) found that oysters stored in wet storage overwinter had higher survival rates and were in better condition than oysters stored in dry storage conditions. Outcomes in the Freeman & Lavoie study (2003) were so clear that the authors recommend that processors who live in the Maritimes in an area close to existing live fish or crustacean holding systems make every attempt to access these facilities to store a portion of their oysters overwinter.

1.3 On land wet storage to prevent dormancy

Instead of allowing newly settled spat to enter dormancy in their first winter, WFA (1977) conducted a study using *Pecten maximus* in an on land recirculating tank system to continue to

allow the newly settled spat to feed and grow in its first winter season. Juveniles were grown in this system in water maintained at 13°C and were fed a supplemental microalgae diet. In this experiment, water flow rates into tanks was an important factor, with growth ceasing completely at flow rates of 12 cm/s and optimal growth occurring in tanks with water flow rates of 0.21cm/s. This study recommends using a larger diameter pipe at the tank bottom to reduce current speeds in overwintering tanks. The WFA (1977) study also demonstrated that algal species and algal density was an important factor in scallop growth. Algal species had different impacts on scallops of different size classes. When compared with spat that were left to overwinter in natural conditions in the sea, the land held scallops had statistically significantly higher weights and were in better condition post winter storage. Additionally, the scallops held in land tanks for their first winter maintained their growth lead in the three months that they were measured during the study. Scallops grown on land during the winter months grew more than twice as fast as scallops that overwintered in the sea. Survival rates in smaller spat sizes were higher in on land overwintered groups than in the scallops overwintered in the sea. In larger size classes survival rates were similar in both overwintered groups. However, upon transfer from on land holding systems to grow out sites in the Spring, mortalities did occur in the on land overwintered group due to changes in water temperature, air exposure and handling stress (WFA 1977). Scallops are much more susceptible to mortalities due to air exposure than the American Oyster.

1.3 Winter Handling

Oysters have a reduced ability to overcome stress events during winter storage, since they are not feeding and have lower respiration rates (Kennedy et al 1996, Morse 2006). As a result, disturbance to oysters held in winter storage should be minimized. Oysters that are being held for sale over the winter months should be held in separate units than oysters that are being held for longterm growout, so that these oysters requiring full winter storage are not stressed by the retrieval of oysters that are being sold during the winter season (Morse 2006). Oysters being held in winter storage also have a reduced ability to repair shell damage,

1.4 Storage timing

Both the timing of placement into storage and the timing of stock removal from storage have major impacts on survival rates in American Oysters held in overwintering storage conditions. Oysters must be placed into storage after they have entered a temperature induced dormancy and be removed from storage before their metabolism is activated again by warming temperatures. Morse (2006) recommends placing oysters into storage when water temperature reaches 2-4°C and removing them from storage when water temperatures go above 4°C. Feeding generally ceases in American oysters when water temperature are reduced below 6°C (Loosanoff 1953, Nelson 1928, Kennedy et al 1996) although research by Comeau et al (2012) has recently shown that oysters in northern climates have adapted to feed at lower temperatures.

1.5 Glycogen Content

A key limiting factor in survivability in bivalve species during long overwintering conditions is the level of glycogen reserves that the bivalves enter the storage season with. Glycogen energy reserves are typically the main energy source for oysters overwintering at low temperatures (Seaman 1991). It has been shown that survivability in Pearl oysters is low when glycogen contents are reduced to 3mg/g near the end of the winter season (Numaguchi 1995). A similar correlation between low survivability and glycogen levels of 2 mg/g was found in Pen Shells in a study by Yurimoto et al (2002). A study by Wang & Amira (1977) found that glycogen reserves were most important to American oysters in the first two months of winter storage.

1.6 Lipid content

Generally, the fluidity of biological membranes is reduced as temperature is reduced. This decrease in membrane fluidity has a negative impact on many cell processes and can lead to mortality events when temperature decreases beyond tolerances. As most poikilotherms do, oysters can restructure the fatty acid components of the membrane as well as the percentage of cholesterol content within the membrane to overcome this decrease in membrane fluidity when water temperature is decreased. However at specific temperatures, American Oyster physiology and behavior becomes limited. At 0°C oysters are typically described as quiescent, at 4°C feeding commences and at 9°C American Oysters typically are able to put energy into growth (Loosanoff 1958). During winter storage, oysters have been shown to have low clearance rates and a lower metabolic rate than other bivalves (*Mytilus edulis* for example). Pernet et al (2007) also found that oysters had low but constant levels of triglycerides throughout winter storage suggesting that they were not heavily utilized by oysters as an energy reserve; whereas triglycerides were an energy source that was used by mussels to survive their overwintering stage. Conversely, Wang & Amiro (1977) found that although glycogen stores were important energy sources for the first two months in storage, lipid reserves were utilized in the later months of winter storage in American Oysters.

1.7 Other factors impacting survivability of oysters through winter storage

1.7.1. Fouling Organisms

Fouling organisms can continue to grow over the winter months, making it critical for growers to overwinter their stock in bay areas or in water systems that do not contain a heavy fouling load. Although oysters are not respiring as actively in winter conditions as during other seasons,

they still require access to fresh, clean water which is more difficult to access when cages are heavily fouled. (Morse 2006). High levels of fouling have been associated with increased winter mortality in some geographic regions.

1.7.2. Spring Food Availability

Most bivalves rely on a combination of lowered metabolic rate and a reliance on internal carbohydrate reserves to survive the overwintering period (Beal et al 2009, Zarnoch & Schreibman 2008). These carbohydrate reserves are usually low by the end of the winter season. While rising Spring temperatures increase the metabolic rates of the bivalves, the energy costs associated with the increased metabolic rates requires an increased level of energy to sustain. This energy is usually accessed through phytoplankton feeding, since internal energy reserves are depleted from the overwintering stage. If, for any reason phytoplankton blooms are delayed or production levels are lower than usual, bivalve mortality can occur. This mortality is often associated with the winter holding period when in actuality it is a Spring mortality event brought on by low phytoplankton levels (Zarnoch & Schreibman 2008, Beal et al 2009).

1.7.3. Winter metabolic rate & release of waste products

Although it has been thought that oysters enter a period of metabolic hibernation at cold temperatures, many studies have shown that oysters do open their valves in winter storage and release waste products and can exhibit low (but present) pumping rates (Loosanoff 1958, Medcof 1958). Wang & Amiro 1977, Seaman & Schwartz 1987, Comeau et al 2012). Wang & Amiro confirmed that oysters do open their valves in cold air storage in addition to in wet storage. In the Wang & Amiro study, the oysters that opened their valves, were more likely to have died by the end of the study, than oysters that held on through the entire storage season with unopened valves. This result suggests that weakened oysters are not able to endure their waste products internally and are forced to open their valves and flush their system to survive. This valve opening is energetically expensive and can use up energy reserves leading to higher mortality rates. Seaman & Schwarz (1987) also measured ammonia levels in tanks for oysters held in wet storage and detected changes in ammonia levels throughout winter storage. Comeau et al (2012) confirmed that oysters held in cold temperatures in northern climates do open their valves and pump at temperatures as low as 0.2°C. These studies indicate that any overwintering storage system must be able to handle metabolic flushing of waste products into the storage system despite the fact that oysters have previously been thought to be metabolically inactive at near freezing water temperatures. Comeau et al (2012) postulate that previous experiments which indicated that oysters are not metabolically active below 5°C were conducted on oysters in the southern and middle distribution range of this species. Comeau et al (2012) focused their study on oysters present in populations in the northern range of this species (Gulf of St. Lawrence, Canada) and found that oysters were opening their valves to a maximum angle of 5.88° in water temperatures that varied between 0.2°C-6.6°C.

1.7.4 Condition prior to winter storage

A significant predictor of oyster survival through winter storage is the condition of oysters prior to placement in any overwintering storage system (Wang & Amiro 1977). Wang & Amiro (1977) found the lowest survival in oysters that entered all overwintering treatments with the lowest condition index.

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