ELSEVIER

Aquaculture 210 (2002) 69–88

Aquaculture

www.elsevier.com/locate/aqua-online

Farming triploid oysters

John A. Nell *

NSW Fisheries, Port Stephens Fisheries Centre, Taylors Beach, NSW 2316, Australia

Received 15 March 2001; accepted 20 September 2001

Abstract

Although the commercial benefits of triploidy have been evaluated in the Pacific oyster *Crassostrea gigas* (Thunberg, 1793), eastern oyster *C. virginica* (Gmelin, 1791), Sydney rock oyster *Saccostrea glomerata* (Gould, 1850) and European flat oyster *Ostrea edulis* (Linnaeus, 1750), so far this technique has only been commercialised for Pacific oysters.

Commercial production of triploids on the West Coast of North America began in 1985. Since then production of triploids has greatly increased and the use of tetraploid males to fertilise eggs from diploids to produce batches of 100% triploids has been developed. In 1999/2000, triploid Pacific oysters made up 30% of all Pacific oysters farmed on the West Coast of North America. Some hatcheries now use tetraploid males instead of chemical or physical stress to produce triploids. The rapid uptake of triploid and tetraploidy techniques has been facilitated by the almost total dependence that these oyster industries have on hatcheries for the supply of seed. This industry in the Pacific Northwest of the US and in British Columbia, Canada, would not have developed to its current size without hatchery seed supplies. Triploids are preferred over diploids in summer because diploids are less marketable when in spawning condition.

There was only limited interest in triploidy production in France until 1999, when IFREMER began to make sperm from tetraploids available to commercial hatcheries. In 1999/2000, only 10% to 20% of all the hatchery-supplied Pacific oyster spat were triploids, but with the use of sperm from tetraploid oysters, this could increase sharply.

Elsewhere around the world, the commercial uptake of triploid oysters has been slow to develop. However, in countries where the production of Pacific oysters is based on hatchery supply of seed, it is likely that with the use of tetraploid oysters, the farming of triploid oysters will increase in the near future.

Keywords: Triploidy; Oysters; Growth; Condition

* Tel.: +61-2-498-212-32; fax: +61-2-498-211-07.
E-mail address: nellj@fisheries.nsw.gov.au (J.A. Nell).

0044-8486/02/$ - see front matter
PII: S0044-8486(01)00861-4

1. Introduction

This review is restricted to the evaluation of triploidy in edible oysters. Many papers have been published since the first paper on triploidy induction in oysters was published by Stanley et al. (1981), and a review on ploidy manipulation in shellfish was published by Beaumont and Fairbrother (1991). Since that last review, triploidy has been induced and evaluated in Pacific *Crassostrea gigas* (Thunberg, 1793), eastern *C. virginica* (Gmelin, 1791), Sydney rock oysters *Saccostrea glomerata* (Gould, 1850) (formerly *S. commercialis*; Buroker et al., 1979; Anderson and Adlard, 1994) and European flat oysters *Ostrea edulis* (Linnaeus, 1750) (see this review). Increases in growth rates and improvements in disease resistance varied widely depending on species, culture conditions and length of experiment. Recent advances in triploidy induction techniques, and the abundance of new literature on triploidy in oysters, justify a review to clarify and summarise this topic for interested farmers and scientists.

The dominant commercial oyster in the Pacific Northwest of the US, the Pacific oyster, undergoes gonad maturation during spring and summer (Breese and Malouf, 1977). The marketability of Pacific oysters in the US drops in summer and autumn when oysters are either gravid (Breese and Malouf, 1977; Allen and Downing, 1991) or spent (Chew, 2000). Therefore, a summer oyster, that is sterile and does not spawn, was developed on the US West Coast by chromosome set manipulation to produce triploidy (Allen and Downing, 1991).

Triploidy in oysters was originally developed in the US to prevent Pacific oysters from reaching spawning condition (Allen and Downing, 1991). In Australia, where Sydney rock oysters are preferably eaten when they have peak meat condition, which is seen usually in oysters in spawning, triploidy was evaluated to determine if the summer marketing season could be extended by preventing oysters from spawning (Nell et al., 1994; Hand et al., 1998a).

2. Triploidy induction

Triploidy can be produced by suppressing meiosis I (MI) or II (MII) (Allen, 1987; Beaumont and Fairbrother, 1991). However, MII triploidy induction results in both higher percentage triploidy and larval survival (Hand et al., 1999). Suppression of MI leads to a high proportion of aneuploids and high mortality (Guo et al., 1992), although MI triploids may grow faster than MII triploids (Stanley et al., 1984). As most reported comparisons between diploids and triploids refer to the use of MII triploids, throughout this review, the term triploid implies an MII triploid, unless otherwise stated. Triploid Pacific oysters are now being produced by crossing tetraploid males with diploid females (Guo et al., 1996), and these triploids grow faster than those MII triploids produced with cytochalasin B (CB) (Wang et al., 1999).

Until the advent of tetraploid oysters (Guo et al., 1996), triploidy induction in oysters was primarily achieved by blocking the release of the second polar body with CB (Allen et al., 1989). However, CB is highly toxic and health concerns may limit its use in food production (Guo et al., 1994). Physical stress, such as heat shock (Quillet and Panelay,

1986; Yamamoto et al., 1988, 1990) and hydrostatic pressure (Chaiton and Allen, 1985; Allen et al., 1986; Shen et al., 1993), has been used to induce triploidy in oysters. However, chemical stress with CB remains more effective (Downing, 1987). In a direct comparison of six methods (CB, heat, calcium, caffeine, combined calcium and heat, and combined caffeine and heat), CB was the most effective overall in producing viable triploids (Scarpa et al., 1994). Desrosiers et al. (1993) and Gérard et al. (1994b) recommended 6-dimethylaminopurine (6-DMAP) as an alternative to CB for triploidy induction in Pacific oysters. Nell et al. (1996) reported that CB treatment resulted in greater survival and higher triploidy percentage than 6-DMAP for triploidy induction in Sydney rock oysters. The optimal conditions and concentrations for triploidy induction with CB in Pacific oysters (Downing and Allen, 1987; Allen et al., 1989), eastern oysters, (Stanley et al., 1981; Barber et al., 1992; Supan et al., 2000) and Sydney rock oysters (Nell et al., 1996) were determined. Those for triploidy induction with 6-DMAP in Pacific oysters were determined by Tian et al. (1999). The use of CB was also successful for triploidy induction in the brooding European flat oyster in which spat were produced by rearing larvae in vitro (Gendreau and Grizel, 1990; Hawkins et al., 1994).

Unfortunately, the physical methods of induction are not always reliable and the chemical methods, in particular those using CB, are costly (Nell et al., 1996) and potentially dangerous to the operator (Guo and Allen, 1994b). Neither physical nor chemical methods can guarantee 100% triploidy. Uniformity in condition (Hand and Nell, 1999) and complete sterility (Guo and Allen, 1994a) in triploids can only be achieved at this time by crossing tetraploids with diploids to produce 100% triploidy (Guo and Allen, 1994b; Guo et al., 1996; Eudeline et al., 2000a,b). A large proportion of triploid Pacific oysters produced in France (Mazurie, personal communication, 2000) and in the US (Chew, 2000) are now produced using tetraploid males to fertilise diploid females. Second-generation tetraploid Pacific oysters have been successfully produced in the US by breeding tetraploid females with tetraploid males (Guo et al., 1996). The future establishment of tetraploid breeding lines would be a significant advance in the commercialisation of the triploidy technique in oysters, as the use of chemicals in the production of both triploids and tetraploids would be unnecessary.

Various methods for tetraploidy induction have been attempted in oysters (Guo et al., 1994; Nell et al., 1998), but the only method successfully used to produce tetraploid oysters is known as ‘blocking polar body 1 in eggs from triploids fertilised by sperm from diploids’ (Guo and Allen, 1994b). This method has successfully produced tetraploid Pacific oysters, which successfully bred to produce second-generation tetraploids (Guo et al., 1996). It has also been used successfully to produce tetraploid eastern oysters (Supan, 2000) and pearl oysters *Pinctada martensi* (Dunker, 1850) (He et al., 2000). This method was refined by Eudeline et al. (2000a,b) who recommended using eggs from individual females rather than eggs pooled from a number of oysters and the use of first polar body as a natural indicator for the duration of chemical application.

The production of tetraploids in Sydney rock oysters has not been successful. Four methods for producing tetraploids were trialed in Sydney rock oysters and while most

produced large numbers of tetraploid larvae, few survived for the past 3 days, and none have survived to settled spat (Nell et al., 1998).

3. Triploidy detection

3.1. Triploidy detection methodology

Direct chromosome counts to determine triploidy levels can only be done readily in day 0 and trochophore larvae (Gérard et al., 1991; Nell et al., 1996). The procedure usually involves arresting cells in metaphase for chromosome counting. It is necessary to allow for chromosome loss and overlapping of cells during slide preparation. Thus, a range of chromosome numbers is used to assign ploidy levels rather than a strict application of chromosome number (Yamamoto et al., 1988; Guo et al., 1992). Due to the difficulties in gaining clear and countable chromosome spreads from shelled larvae, spat and adult oysters, flow cytometry is often the preferred method for determining the ploidy level in these life stages (Allen, 1983). Flow cytometry measures the fluorescence of the cell nucleus after it has been stained with a nucleic acid specific dye, either propidium iodide (PI) (Utting and Child, 1994) or 4′ ,6-diamidino-2phenylindole (DAPI) (Chaiton and Allen, 1985). The amount of dye taken up by the cell in a saturated solution of the dye is generally proportional to the amount of DNA in the nucleus. Somatic cells of triploid oysters will therefore emit 1.5 times the fluorescence of diploid cells.

Several other methods for the determination of ploidy level exist including image analysis (Gérard et al., 1994a), microfluorometry (Komaru et al., 1988; Uchimura et al., 1989; Durand et al., 1990) and nuclear sizing (Child and Watkins, 1994; Gardner et al., 1996). Image analysis measures the optical density of stained nuclei, microfluorometry is in principle similar to flow cytometry and nuclear sizing involves comparison of the nuclear size of gill tissue and haemolymph cells between diploids and triploids.

3.2. Mosaicism and triploidy reversion

Mosaics (2*n*/3*n*) have been found in the batches of triploid oysters in Pacific oysters (Allen, 1994; Guo and Allen, 1994a; Allen et al., 1996, 1999; Chandler et al., 1999), Suminoegaki oysters *C. ariakensis* (Fujita, 1913) (Chandler et al., 1999) and Sydney rock oysters (Hand et al., 1999). Reversion of triploids to mosaics or diploids was reported in Pacific oysters (Allen, 1994; Allen et al., 1996, 1999). Hand et al. (1999) reported that 12–47% of nearly 3-year-old putative triploid Sydney rock oysters were in fact triploid/diploid mosaics. Reductions in triploidy levels of $\geq 20\%$ have been reported in Pacific oysters (Allen et al., 1996; Kent, personal communication, 2000). It is not known if triploidy reversion also occurs in Sydney rock oysters. In fact, over the 10 years of experimentation from 1989 to 1998, the triploidy level from 3-day-old larvae to metamorphosis and through settlement to spat and market size has always increased (Nell, unpublished data, 2000). It is thought that this happens because of better survival of triploids. This phenomenon would have masked any triploidy reversion that may have occurred in Sydney rock oysters.

4. Growth

4.1. Pacific oysters, C. gigas

Faster growth rates were reported for both chemically induced triploid Pacific oysters (Fig. 1) (Allen and Downing, 1986) as well as triploids produced from tetraploids (Guo et al., 1996). Although, in good growing areas, triploids grow faster than diploids, in poor ones, there is no difference in growth rate between these two types of oysters (Thompson, personal communication, 2000). Relatively small increases in growth rates have been reported for triploid Pacific oysters grown at high latitudes (42–43 °S) such as in Tasmania, Australia (Maguire et al., 1994a), but at warmer/lower latitudes (33–34 °N) such as in Hiroshima, Japan, triploid Pacific oysters grow much faster than diploids (Table 1). Akashige and Fushimi (1992), showed an 81% increase in whole weight (Table 1) for Pacific oysters in Hiroshima Bay after an experimental period of only 8 months. A similar observation was made in a laboratory study with Pacific oysters by Shpigel et al. (1992), who also reported faster growth rates at 30 °C when compared to oysters held at 8–15 °C. A study by Chao et al. (1999), who measured shell height rather than the whole weight, also showed superior growth for triploid oysters in Taiwan compared with that of diploids.

4.2. Sydney rock oysters, S. glomerata

Sydney rock oysters are cultivated in Australia (Nell, 1993). In NSW, Australia, triploids reach market size (Fig. 2) (40–60 g whole weight) on average 6 months earlier

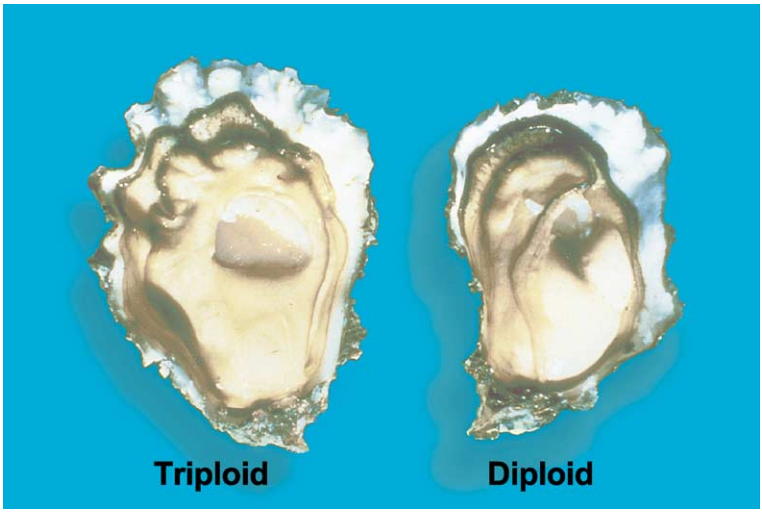

Fig. 1. Triploid and diploid sibling Pacific oysters *C. gigas* for the half-shell trade. Photograph by Terry Nosho, University of Washington, Seattle, WA.

Table 1
Comparative performance of diploid and MII triploid oysters

Species	Country/ region	Latitude	Length of experiment (months)	Number of sites tested	Average whole weight of oysters (g)				Increase in weight of triploids as compared to diploids (%)	Reference
					Start of experiment		End of experiment			
					Dip	Trip	Dip	Trip		
Pacific oysters *Crassostrea gigas*	Japan	33–34°N	8	4	5	6	43	78	81	Akashige and Fushimi, 1992
Pacific oysters	Australia (Tasmania)	42–43°S	27	2	<1	<1	91	105	15	Maguire et al., 1994a
			42	1	<1	<1	101	108	7	
Eastern oyster *Crassostrea virginica*	US East coast	36–38°N	17	1	1	3	41	53	29	Barber and Mann, 1991
Eastern oyster	US East coast	40–41°N	17	3	<1	<1	31	78	60	Matthiessen and Davis, 1992
Sydney rock oysters *Saccostrea glomerata*	Australia (NSW)	32–33°S	29	4	<1	<1	39	55	41	Nell et al., 1994
Sydney rock oysters	Australia (NSW)	31–37°S	28	13	<1	<1	28	37	31	Hand et al., 1998a

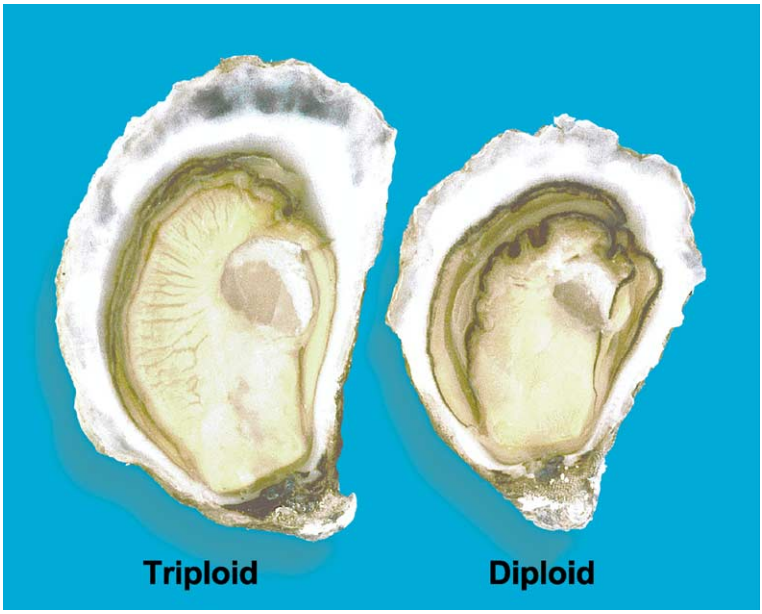

Fig. 2. Triploid and diploid sibling Sydney rock oysters *S. glomerata*. Oysters are 3 years old, the whole weight of the diploid is around 40 g and that of the triploid around 50 g. Photograph by Ian R. Smith, NSW Fisheries, Taylors Beach, NSW.

than the usual 3.5 years for diploids (Nell et al., 1994; Hand et al., 1998a; Nell and Maguire, 1998). Although results vary between farms, after 2–2.5 years on commercial oyster leases triploids are on average 30.7% heavier and 8.6% larger in shell height than sibling diploids (Hand et al., 1998a). The growth advantage of triploids seems to be expressed at specific sizes rather than ages, specifically at a mean whole weight above 5–10 g or shell height of 30–40 mm. In general, triploids appear to grow faster relative to diploids at higher water temperatures (Hand et al., 1998a) (i.e. lower latitudes in Australia). Preliminary data have shown that the percentage weight increase in the triploid progeny of both non-selected 'wild' oysters and third-generation breeding lines selected for fast growth is similar compared to their respective diploid siblings (Hand, unpublished data, 2001). This suggests that, as expected, the growth advantages of triploidy and selective breeding are additive, as they are achieved through different means. This experiment was carried out by comparing the growth of diploid and triploid siblings of the most advanced Sydney rock oyster breeding line with that of controls.

4.3. Eastern oysters, C. virginica

Barber and Mann (1991) reported that triploid eastern oysters reached market size of 60 mm shell height or 50 g whole weight (Table 1), 5 months earlier out of a growing period of 17 months as compared to diploids. That is the triploids were on average 29% heavier

(whole weight) at the end of the study period in November 1990. This result was supported by a similar finding by Mathiessen and Davis (1992), who reported that triploids were on average 60% heavier after a 17-month experimental period. In eastern oysters, the superior growth of triploids becomes more pronounced in the second season (Stanley et al., 1981; Barber and Mann, 1991; Matthiessen and Davis, 1992), presumably because of an increased gonad development in 2-year-old diploid oysters.

4.4. Why triploids grow faster than diploids

Although triploids grow faster, there is more variation in growth among triploids than in diploids (Goulletquer et al., 1995). They grow faster at least partly because of energy reallocation from gametogenesis to growth (Allen and Downing, 1986; Barber and Mann, 1991; Hawkins et al., 1994; Hand et al., 1998a). This faster growth becomes more pronounced after the first year (Stanley et al., 1984; Barber and Mann, 1991; Hand et al., 1998a), when diploids become more sexually active. Although, energy reallocation does not explain why triploids grow faster than diploids before sexual maturation (Jiang et al., 1991), it is certainly important as in poor growing areas that there is no difference in growth rate between these two types of oysters (Thompson, personal communication, 2000).

Increased heterozygosity in both MI and MII triploids may also contribute to the faster growth of both MI and MII triploids (Allendorf and Leary, 1984). MI triploid eastern (Stanley et al., 1984), Pacific (Yamamoto et al., 1988) and European flat oysters (Hawkins et al., 1994) grow faster than MII triploids. It was suggested (Stanley et al., 1984; Beaumont and Kelly, 1989; Hawkins et al., 1994) that the faster growth of MI as compared to MII resulted from their increased heterozygosity as measured over several protein loci, i.e. multiple-locus heterozygosity (MLH). However, Guo and Allen (1994c) suggested that the MLH of MI triploids may actually be lower than that of MII triploids, depending on meiotic crossover frequency. Other studies also showed a lack of correlation between MLH and growth in MI and MII triploids (Jiang et al., 1991; Mason et al., 1988). However, the correlation between MLH and growth rate in natural diploid populations is rather weak (Gaffney, 1990).

Guo and Allen (1994c) agreed that both heterozygosity and energy reallocation may contribute positively to the overall performance of triploids. However, they also suggested the hypothesis of polyploid gigantism, in which the increased cell volume and lack of cell-number compensation is the main reason for faster growth in triploids. Polyploid gigantism in triploid Pacific oysters produced from tetraploids was observed by Guo et al. (1996) and demonstrated by Wang et al. (2001). Guo and Allen (1994c) also postulated that environmental factors such as nutrition may have an impact on polyploids. They speculated that because triploid cells are larger, they may need more nutrients to grow and divide. Therefore, in an environment where food supplies are limited, triploids may not be larger than diploids before sexual maturation (Guo and Allen, 1994c). They also stated that the heterozygosity and energy reallocation hypotheses are insufficient in explaining the faster growth of triploids, and that the polyploidy gigantism hypothesis needs to be tested by direct studies on cell size, cell number and organ size in diploids and triploids.

Aneuploidy may be a cause of slow growth in oysters (Thiriot-Quiévreux et al., 1988, 1992; Zouros et al., 1996; Leitão et al., 2001). Zouros et al. (1996) proposed that the inverse relationship between aneuploidy and growth is due to the unmasking of deleterious recessive genes caused by 'progressive haploidisation' of somatic cells. Zouros et al. (1996) further suggested that the probability that the chromosome loss will expose a recessive deleterious mutation is much smaller in triploids than in diploids.

The hypotheses of heterozygosity, energy reallocation, polyploidy gigantism and reduction of any possible negative effects of aneuploidy are all plausible and may all contribute to faster growth of triploids at times. Further research is required to determine the relative importance of these putative factors.

5. Condition and gonad development

5.1. Pacific oysters, C. gigas

Triploidy was developed in Pacific oysters in the US to reduce gonad development during spring and summer (Allen and Downing, 1991). Triploid Pacific oysters show reduced gonadogenesis and a slower reduction in glycogen as compared to diploids over the spawning season (Allen and Downing, 1986; Akashige, 1990; Akashige and Fushimi, 1992). Relative to diploids, gametogenesis in triploids was retarded but not absent in Humbolt Bay, CA (40–41°N) (Allen and Downing, 1990) and in Tasmania, Australia (42–43°S) (Gardner et al., 1994). Both studies reported that the male to female sex ratio in triploids was similar to that in diploids, but the proportions of hermaphrodites in the triploids in California (Allen and Downing, 1990) and Tasmania (Gardner et al., 1994) were different at 29% and 3%, respectively. Allen and Downing (1990) in their Californian study reported that gametogenesis was more retarded in females than in males, whereas Gardner et al. (1994) showed the reverse in Tasmania. The reproductive potential of a triploid Pacific oyster population in which sperm from triploid males fertilised eggs from triploid females was estimated to be only 0.0008% of that of normal diploids in a laboratory study in which oysters were strip spawned (Guo and Allen, 1994a). It is likely therefore that in a natural situation the reproductive potential of triploids is even lower (Guo and Allen, 1994a).

5.2. Sydney rock oysters, S. glomerata

For meat condition in Sydney rock oysters, the relative performance in terms of triploids compared to diploids varied among five widely distributed sites (31–37°S) in New South Wales (Hand and Nell, 1999). Over the final year of the study when oysters were reaching market size (40–60 g whole weight), condition indices of triploids were higher or not different to those of diploids throughout the year except for summer, when diploids were at the peak of their condition (Hand and Nell, 1999). As Sydney rock oysters are preferably eaten when in spawning condition, diploids are the recommended crop for summer, and triploids for winter. Although triploid Sydney rock oysters may exhibit brown discolouration of the gonad surface, this problem is less noticeable during the

cooler months, when their meat condition is superior to that of diploids (Hand and Nell, 1999).

The extent of retardation of gametogenesis in triploid Sydney rock oysters is more severe than in triploid Pacific oysters (Cox et al., 1996). Sydney rock oyster triploids are functionally sterile (Cox et al., 1996), whereas evidence of spawning was observed in triploid Pacific oysters on the West Coast of the US (Allen and Downing, 1990) and in Tasmania, Australia (Gardner at al., 1994). Another major difference between the Sydney rock and Pacific oysters was the frequency of reported hermaphrodites. Cox et al. (1996) reported only 1.3% hermaphrodites in Sydney rock oyster triploids whereas Allen and Downing (1990) observed 29% hermaphrodites in Pacific oyster triploids. In contrast, Gardner et al. (1994), recorded only 3% hermaphrodites in triploid Pacific oysters. The proportion of hermaphrodites in diploid Pacific (Gardner et al., 1994), and Sydney rock (Cox et al., 1996) oysters of $<2\%$ is also quite low.

6. Consumer acceptance

Sensory comparisons of diploids and triploids by taste panels for Pacific oysters (Allen and Downing, 1991; Maguire et al., 1994a; Chao et al., 2001) and Sydney rock oysters (Korac et al., 1996) all showed high consumer acceptance of triploids. However, anecdotal comments from oyster farmers and processors suggest that there may be a buyer resistance to both triploid Pacific and Sydney rock oysters when diploids are in peak spawning condition in summer.

The superior flavour of triploids was attributed to their higher glycogen content (Allen and Downing, 1991). However, as Maguire et al. (1994a) reported that glycogen is tasteless, the taste preference for triploids reported by Allen and Downing (1991) may have resulted from the firmer texture of the triploids as compared to the softer texture of the gravid diploids.

7. Discolouration

Discolouration of triploid oysters has been reported in Pacific oysters by Maguire et al. (1994b), who noted that 6% of triploid Pacific oysters grown in Tasmania (but not diploids) developed brown patches on the meats in summer. A similar effect was seen by Hand and Nell (1999) in Sydney rock oysters, which were particularly prone to localised discolouration of the gonad. This discolouration differed from the grey gonad patchiness frequently seen in diploids following partial spawning. Rather, triploid Sydney rock oysters developed distinct pale to dark brown patches on the gonad surface. Triploid Pacific oysters produced from tetraploids also have discolouration in some, but not all, populations (Guo, personal communication, 2000).

Discolouration of Sydney rock and Pacific oysters appeared to increase during the warmer months of the year, particularly during the post-spawning season of diploids, and was generally less obvious during winter/spring (Maguire et al., 1994a; Hand and Nell, 1999). The discolouration was not correlated with condition index in triploids (Hand and

Nell, 1999). Limited literature is available on meat colour in oysters. Live eastern oysters form brown cells in response to contaminant exposure and stress (Zaroogian and Yevich, 1993). These cells are possibly involved in detoxification and range in colour from light to black-brown in oysters from polluted sites. Since brown cells are associated with connective tissue rather than with gonad tissue, it is unlikely that they are responsible for discolouration in triploids.

Meat discolouration is a common problem with shucked and processed oysters. Dried (Choi et al., 1977) and frozen (Jeong et al., 1990) oysters develop brown discolouration due to oxidative rancidity of lipids. High storage temperatures of canned oyster meat are also known to cause brown colouration due to the non-enzymatic browning 'Maillard' reaction between glucose and amino acids (Lee et al., 1976). Differences in protein metabolism, due to reduced energy expenditure associated with lower concentrations of RNA per unit total tissue protein, of MI triploids compared to diploid oysters, have been detected in the European flat oyster, (Hawkins et al., 1994). Shpigel et al. (1992) reported the elevated tissue dry weight, condition index, carbohydrate and protein levels for triploid Pacific oysters held at high (30 °C) temperatures as compared to diploids. Further investigation is required to determine whether the higher protein levels (hence, different free amino acid equilibria) in triploid oysters combined with high temperatures during summer could cause a browning effect on the gonad (Hand and Nell, 1999).

8. Disease resistance

In general, Pacific oysters seem to be resistant to disease and associated mortalities caused by protistan parasites of other oyster species (Elston, 1993; Bower et al., 1994; Burreson et al., 2000). Allen and Downing (1986) reported that triploid Pacific oysters have a higher survival rate than diploids, but they did not specify the cause of mortality. Meyers et al. (1991), who exposed diploid and triploid Pacific and eastern oysters to the *Perkinsus marinus* parasite, reported that the Pacific oysters were more tolerant of *P. marinus* than eastern oysters, but that triploidy provided no increased disease tolerance for either species. Triploid eastern oysters suffered lower mortality from MSX (*Haplosporidium nelsoni*) than diploids (Mathiessen and Davis, 1992). However, Cheney et al. (2000) reported that the summer mortality in Pacific oysters, which is most likely caused by chronic stress, killed more triploids than diploids. Lower survival in triploid Pacific oysters in high carrying capacity estuaries was reported by Goulletquer et al. (1995). Sami et al. (1991) investigated the possible cause for the higher survival of triploids Pacific oysters and found they have more haemocytes with Concanavalin-A binding sites, associated with increased phagocytosis (Moore and Gelder, 1987), than diploids.

Sydney rock oysters suffer from two major parasites, *Mikrocytos roughleyi* (Farley et al., 1988) and *Marteilia sydneyi* (Perkins and Wolf, 1976), which cause the diseases winter mortality and QX disease, respectively. Hand et al. (1998a,b) reported that death from winter mortality is reduced by more than half in triploid oysters when compared to diploid oysters. Triploid Sydney rock oysters have not as yet been exposed to QX disease and it remains to be seen as yet is unknown if triploid Sydney rock oysters have tolerance to this parasite. Generalised statements about differential survival of diploid and triploid oysters

cannot be made as the interaction between disease and or stressor with ploidy level is different for each situation and oyster species.

9. Commercialisation of triploid oysters

9.1. North America

The Pacific oyster industry in North America is large, and in 1999/2000, it produced approximately 5000 t of oyster meat or 33,000 t whole weight of oysters (Nosho, personal communication, 2000). Because of the unreliability of natural catch, this industry was quick to adapt hatchery technology and the use of natural catch is now insignificant (Lindsay and Simons, 1997). Commercial production of triploid Pacific oysters *C. gigas* on the West Coast of America began in 1985 (Allen et al., 1989). Since then, the production of triploids has greatly increased and the use of tetraploid males to fertilise eggs from diploids to produce batches of 100% triploids has been introduced (Chew, 2000; Cudd, personal communication, 2000). Shellfish hatcheries in Washington and Oregon are currently (1999/2000) producing about 37.5 billion ready-to-set or 'eyed' larvae each year, of which 12 billion or about 1/3 are triploid (Nosho, personal communication, 2000; Thompson, personal communication, 2000). Farmers believe that triploid larvae are harder to set than diploids (Thompson, personal communication, 2000).

This use of sterile triploid Suminoe oyster *C. ariakensis* (Fugita, 1919) is being assessed on both the West Coast (Langdon and Robinson, 1996) and the East Coast (Calvo et al., 1999, 2000; Allen, 2000) as a means of evaluating the performance of non-native bivalves. This novel use of triploids may be used for other bivalves in other places to test their performance while preventing their release into the environment.

9.2. Europe

The main species farmed in France is the Pacific oyster where five hatcheries supplied 10–15% of the seed requirement for this species in 1999/2000, with the remainder coming from natural catch (Gerard, personal communication, 2000). There was only limited interest in triploidy production in France until IFREMER began to make sperm from tetraploids available to commercial hatcheries (Gerard, personal communication, 2000). In 1999/2000, only 10% to 20% of all hatchery-supplied Pacific oyster spat were triploids, but with the use of sperm from tetraploid oysters, it could soon reach 50% (Le Borgne, personal communication, 2000). In 1998/1999, there was only one hatchery selling triploid seed, produced by using CB in Europe, but now there are four hatcheries in France buying sperm from IFREMER (Le Borgne, personal communication, 2000). Triploid oysters can be sold on the market at any time because their taste is usually judged superior, but there is a special interest in them during the summer months from June to September—i.e. the spawning season for Pacific oysters in France (Le Borgne, personal communication, 2000). The proportion of triploid oysters coming from hatcheries is increasing as farmers gain confidence in the reliability of this source (Gerard, personal communication, 2000; Le Borgne, personal communication, 2000).

The experience with triploid Pacific oysters in the UK has been disappointing, as the highest level of triploidy obtained using CB was only 70%, growth rates were not improved and meat yields were consistently poor (Bayes, personal communication, 2000). However, hatcheries in the UK are keen to try again with the purchase of sperm from tetraploid oysters (Bayes, personal communication, 2000).

9.3. Australia

Scientific evaluations of triploid Pacific oysters in Tasmania, Australia (42–43°S) (Table 1), reported faster growth for triploids after they reached market size (60–80 g whole weight) and maintenance of better meat condition over the summer/autumn spawning period after oysters had reached market size (Maguire et al., 1994b). Taste tests showed that consumer acceptability of triploids was high, but Maguire et al. (1994a) reported a 6% incidence of brown patches on the meats of triploids and none on diploid controls during summer. Two experimental batches of triploids were produced for farmers in Tasmania and South Australia. The experience of farmers with triploid oysters in South Australia (32–35°S) was similar to that in Tasmania; but the superior meat condition of triploids in autumn after diploids had spawned out was considered to be an important marketing advantage for South Australian farmers (Zippel, personal communication, 2000).

Despite the conflict between promising scientific reports (Maguire et al., 1994a,b) and a rather negative initial industry reaction, there is still some interest in trying triploid Pacific oysters again. In Australia, one of the biggest oyster hatcheries in Tasmania has a target of 2 million triploids which is <2% of the company's total production of oyster spat to be sold to farmers in Tasmania and South Australia in 2000/2001 (Pugh, personal communication, 2000).

Staff from the University of Tasmania produced, using the method by Guo and Allen (1994b), a batch of tetraploid oyster spat (20% tetraploidy) in 1997. Tetraploid males were used successfully to fertilise eggs from diploids to produce experimental batches of 100% triploid oysters (Thompson, personal communication, 2000). However, attempts to produce second-generation tetraploids have failed so far (Thompson, personal communication, 2000). The development of 100% triploid Pacific oysters in Australia would have great advantages, beyond extending the marketing season in autumn: it might facilitate the approval for Pacific oyster farming in potentially large growing areas in Tasmania and other states that have been denied to farmers, partly because of concerns about the problems with Pacific oyster settlement on the foreshores and competition with endemic oysters such as Sydney rock oysters.

Sydney rock oyster triploids performed very well in NSW, Australia. Unfortunately, despite excellent results, there has been limited commercial uptake of triploid technology in NSW, Australia. Farmers are keen to try triploid oysters, and many have been supplied small batches of 50,000–250,000 spat by the NSW Fisheries hatchery at the Port Stephens Fisheries Centre. Those that have tried them are keen to buy more, but until the production problems of early larval mortality around (2–8 days of age) and post-settlement mortality of spat (0.5–2.0 mm shell height at 1–6 weeks of age) have been resolved, commercial production of both diploid and triploid Sydney rock oysters remains problematic (Heasman et al., 2000).

9.4. Asia

Triploid Pacific oysters have been evaluated in Taiwan (Chao et al., 1999, 2001), Japan (Komaru, personal communication, 2000) and China (Wang, personal communication, 2000). Triploid Pacific oysters have been in commercial production in China at least since 1997 and pilot scale testing of tetraploids for triploid production commenced in 2000 (Guo, personal communication, 2000).

10. Summary

So far, the triploidy technique has been commercially adapted only in Pacific oysters. While triploidy induction was restricted to the use of physical, in particular hydrostatic pressure and chemical stress (CB), large scale triploid farming was restricted to the West Coast of North America. However, with the development of tetraploid oysters, commercial production of triploid oysters has increased in France and China, with other countries likely to follow this example. The production of tetraploid eastern oysters (Supan, 2000) may promote the interest in the use of triploidy in this species.

The main advantage of farming triploid oysters in North America and Europe is that they do not come into a spawning condition during summer, and thus retain their marketability all year round. In Australia, where Pacific oysters in spawning condition are readily accepted by consumers, farmers desire triploids as a winter crop to be marketed when diploids have spawned out.

Triploid eastern and Sydney rock oysters grow much faster than diploids, and triploid Sydney rock oysters suffer half the mortality of the diploids from one of their major diseases, winter mortality. However, commercial adaption of the triploidy technique in both these two species is still a long way off. Both the eastern and Sydney rock oyster industries are largely reliant on natural catch for their spatfall, and it will be a slow process for farmers to change both their source of spat as well as adapt different nursery and growing techniques to take advantage of hatchery seed. The Sydney rock oyster is the species that benefits most from triploidy by faster growth, an alternative crop to diploids over autumn and winter when the latter have lost meat condition, and partial tolerance to one of its major diseases. However, until hatchery production problems associated with high larval mortality and post-settlement mortality have been overcome, large-scale production of Sydney rock oyster spat is not feasible (Heasman et al., 2000).

11. The future

Beyond the broader adaptation of triploid technology, there are likely to be several changes to existing techniques. The use of current chemical methods, especially CB, for the induction of triploidy in oysters is likely to be limited in the future because of its dangers to operators, and variable results. Instead, the use of tetraploids to produce triploids is likely to continue and increase. This will be facilitated by the use of tetraploid breeding lines rather than the continued chemical production of tetraploids. Second-

generation Pacific oyster tetraploids have already been produced by mating tetraploid males and females (Guo et al., 1996). Once tetraploid breeding lines have been established they, and the diploid breeding lines, can each be selectively bred so that their progeny will have characteristics of value to oyster farmers. Sperm from tetraploid males provide two thirds of the genetic complement of their triploid progeny, so selecting the tetraploid lines as well as the diploid breeding lines is of obvious importance. In Australia, the faster growth and improved survival of triploid Sydney rock oysters has large commercial potential. Thus, the improved version of the only successful method for tetraploidy induction 'blocking polar body 1 in eggs from triploids fertilised by sperm from diploids, using first polar body as a natural indicator' (Eudeline et al., 2000a,b) should be tested on this species. The benefits of triploidy can only be increased by the production of triploids through the use of sperm from tetraploids to fertilise eggs from diploids.

Acknowledgements

Special thanks are due to Mr. Terry Nosho, University of Washington for supplying the information on the oyster industry in the Pacific North West of the US. I would also like to thank Ms. Rosalind Hand, Dr. Wayne O'Connor and Mr. Ian Smith NSW Fisheries and Dr. Ximing Guo, Haskin Shellfish Research Laboratory for their comments on this review. I would particularly like to thank colleagues who contributed to this review by means of personal communication.

References

Akashige, S., 1990. Growth and reproduction of triploid Japanese oysters in Hiroshima Bay. In: Hoshi, M., Yamashita, O. (Eds.), Proc. 5th Int. Congress Invertebrate Reproduction, Nagoya, 1989. Adv. Invertebr. Reprod., vol. 5. Elsevier, Amsterdam, pp. 461–468.

Akashige, S., Fushimi, T., 1992. Growth, survival and glycogen content of triploid Pacific oyster *Crassostrea gigas* in the waters of Hiroshima, Japan. Nippon Suisan Gakkaishi 58, 1063–1071.

Allen Jr., S.K., 1983. Flow cytometry: assaying experimental polyploid fish and shellfish. Aquaculture 33, 317–328.

Allen Jr., S.K., 1987. Genetic manipulations—critical review of methods and performances for shellfish. Proc. World. Symp. On Selection, Hybridization, and Genetic Engineering in Aquaculture, Bordeaux, 27–30 May 1986, vol. II. European Inland Fisheries Advisory Commission of FAO (EIFAC) and International Council for the Exploration of the Seas (ICES), Berlin, pp. 127–134.

Allen Jr., S.K., 1994. Estimating the ecological impact of triploid *Crassostrea gigas*: what is the meaning of sterility? FASIP Leave Report, Unité de Recherche en Génétique et Encloserie, IFREMER, La Tremblade, France, 33 pp.

Allen Jr., S.K., 2000. Research and development on Suminoegaki, *Crassostrea ariakensis*, for aquaculture in Virginia, and other activities with non-natives. J. Shellfish Res. 19, 612 (abstract).

Allen Jr., S.K., Downing, S.L., 1986. Performance of triploid Pacific oysters, *Crassostrea gigas* (Thunberg): I. Survival, growth, glycogen content, and sexual maturation in yearlings. J. Exp. Mar. Biol. Ecol. 102, 197–208.

Allen Jr., S.K., Downing, S.L., 1990. Performance of triploid Pacific oysters, *Crassostrea gigas*: gametogenesis. Can. J. Fish. Aquat. Sci. 47, 1222–1232.

Allen Jr., S.K., Downing, S.L., 1991. Consumers and experts alike prefer the taste of sterile triploid over gravid diploid Pacific oysters (*Crassostrea gigas*, Thunberg, 1793). J. Shellfish Res. 10, 19–22.

Allen Jr., S.K., Downing, S.L., Chaiton, J., Beattie, J.H., 1986. Chemically and pressure-induced triploidy in the Pacific oyster *Crassostrea gigas*. Aquaculture 57, 359–360 (abstract).

Allen Jr., S.K., Downing, S.L., Chew, K.K., 1989. Hatchery Manual for Producing Triploid Oysters. University of Washington Press, Seattle, WA, 27 pp.

Allen Jr., S.K., Guo, X., Burreson, G., Mann, R., 1996. Heteroploid mosaics and reversion among triploid oysters, *Crassostrea gigas*. Fact or artifact. J. Shellfish Res. 15, 514 (abstract).

Allen Jr., S.K., Howe, A., Gallivan, T., Guo, X., DeBrosse, G., 1999. Genotype and environmental variation in reversion of triploid *Crassostrea gigas* to the heteroploid mosaic state. J. Shellfish Res. 18, 293 (abstract).

Allendorf, F.W., Leary, R.F., 1984. Heterozygosity in gynogenetic diploids and triploids estimated by gene-centromere recombinations. Aquaculture 43, 413–420.

Anderson, T.J., Adlard, R.D., 1994. Nucleotide sequence of a rDNA internal transcribed spacer synonymy of *Saccostrea commercialis* and *S. glomerata*. J. Mol. Stud. 60, 196–197.

Barber, B.J., Mann, R., 1991. Sterile triploid *Crassostrea virginica* (Gmelin, 1791) grow faster than diploids but are equally susceptible to *Perkinsus marinus*. J. Shellfish Res. 10, 445–450.

Barber, B.J., Mann, R., Allen Jr., S.K., 1992. Optimization of triploid induction for the oyster *Crassostrea virginica* (Gmelin). Aquaculture 106, 21–26.

Beaumont, A.R., Fairbrother, J.E., 1991. Ploidy manipulation in molluscan shellfish: a review. J. Shellfish Res. 10, 1–18.

Beaumont, A.R., Kelly, K.S., 1989. Production and growth of triploid *Mytilus edulis* larvae. J. Exp. Mar. Biol. Ecol. 132, 69–84.

Bower, S.M., Hervio, D., McGladdery, S.E., 1994. Potential for the Pacific oyster, *Crassostrea gigas*, to serve as a reservoir host and carrier of oyster pathogens. International Council for the Exploration of the Sea, ICES-CM-1994/F:30 (ICESCM1994F30), 5 pp.

Breese, W.P., Malouf, R.E., 1977. Hatchery rearing techniques for the oyster *Crassostrea rivularis* Gould. Aquaculture 12, 123–126.

Burreson, E.M., Stokes, N.A., Friedman, C.S., 2000. Increased virulence in an introduced pathogen: *Haplosporidium nelsoni* (MSX) in the eastern oyster *Crassostrea virginica*. J. Aquat. Anim. Health 12, 1–8.

Buroker, N.E., Hershberger, W.K., Chew, K.K., 1979. Population genetics of the family Ostreidae: I. Intraspecific studies of *Crassostrea gigas* and *Saccostrea commercialis*. Mar. Biol. 54, 157–169.

Calvo, G.C., Luckenbach, M.W., Burreson, E.M., 1999. Evaluating the performance of non-native oyster species in Virginia. J. Shellfish Res. 18, 303 (abstract).

Calvo, G.C., Luckenbach, M.W., Burreson, E.M., 2000. High performance of *Crasssostrea ariakensis* in Chesapeake Bay. J. Shellfish Res. 19, 643 (abstract).

Chaiton, J.A., Allen Jr., S.K., 1985. Early detection of triploidy in the larvae of Pacific oysters, *Crassostrea gigas* by flow cytometry. Aquaculture 48, 35–43.

Chandler, W., Howe, A., Allen Jr., S.K., 1999. Mosaicism of somatic and gametic tissues in *Crassostrea gigas* and *C. ariakensis*. J. Shellfish Res. 18, 29 (abstract).

Chao, N.H., Tsai, H.P., Liang, C.I., Chen, H.Y., Cheng, J.H., Ho, Y.T., 1999. Induction and performance of triploid oyster, *Crassostrea gigas*, in Taiwan. COA (Council of Agriculture, Taiwan) Fisheries Series No. 65, pp. 99–115 (In English with Chinese abstract).

Chao, N.H., Cheng, J.H., Liang, C.I., Tsai, H.P., Chen, H.Y., Ho, Y.T., 2001. Determination of induced triploid oysters on its preliminary extension in Taiwan. In: Liao, I.C., Baker, J. (Eds.), Proceedings of the Joint Taiwan Australia Aquaculture and Fisheries Resource and Management Forum, Nov. 2–8, 1998. Taiwan Fisheries Research Institute, Keelung, Taiwan.

Cheney, D.P., MacDonald, B.F., Elston, R.A., 2000. Summer mortality of Pacific oysters, *Crassostrea gigas* (Thunberg): initial findings on multiple environmental stressors in Puget Sound, Washington, 1998. J. Shellfish Res. 19, 353–359.

Chew, K.K., 2000. Update on triploid Pacific oysters. Aquacult. Mag. 26 (4), 87–89.

Child, A.R., Watkins, H.P., 1994. A simple method to identify triploid molluscan bivalves by the measurement of cell nucleus diameter. Aquaculture 125, 199–204.

Choi, J.H., Lee, K.H., Kim, M.N., 1977. Inhibition of browning reactions occurring in the storage of dried oyster: 2. Inhibitory effect of sodium sulfite treatment and the addition of antioxidants. Bull. Korean Fish. Soc. 10, 17–22.

Cox, E.S., Smith, M.S.R., Nell, J.A., Maguire, G.B., 1996. Studies on triploid oysters in Australia: VI. Gonad development in diploid and triploid Sydney rock oysters *Saccostrea commercialis* (Iredale and Roughley). J. Exp. Mar. Biol. Ecol. 197, 101–120.

Desrosiers, R.R., Gérard, A., Peignon, J.M., Naciri, Y., Dufresne, L., Morasse, J., Ledu, C., Phelipot, P., Guerrier, P., Dubé, F., 1993. A novel method to produce triploids in bivalve molluscs by the use of 6-dimethylaminopurine. J. Exp. Mar. Biol. Ecol. 170, 29–43.

Downing, S.L., 1987. Induction of triploidy in Pacific oyster (*Crassostrea gigas*) using cytochalasin B and hydrostatic pressure: optimal treatments depend on temperature. MS Thesis, University of Washington, Seattle, WA, 126 pp.

Downing, S.L., Allen Jr., S.K., 1987. Induced triploidy in the Pacific oyster, *Crassostrea gigas*: optimal treatments with cytochalasin B depend on temperature. Aquaculture 61, 1–15.

Durand, P., Wada, K.T., Komaru, A., 1990. Triploidy induction by caffeine-heat shock treatments in the Japanese pearl oyster *Pinctada fucata martensii*. Nippon Suisan Gakkaishi 56, 1423–1425.

Elston, R.A., 1993. Infectious diseases of the Pacific oyster, *Crassostrea gigas*. Ann. Rev. Fish Dis. 3, 259–276.

Eudeline, B., Allen Jr., S.K., Guo, X., 2000a. Optimization of tetraploid induction in Pacific oysters, *Crassostrea gigas*, using first polar body as a natural indicator. Aquaculture 187, 73–84.

Eudeline, B., Allen Jr., S.K., Guo, X., 2000b. Delayed meiosis and polar body release in eggs of triploid Pacific oysters, *Crassostrea gigas*, in relation to tetraploid production. J. Exp. Mar. Biol. Ecol. 248, 151–161.

Farley, C.A., Wolf, P.H., Elston, R.A., 1988. A long-term study of "Microcell" disease in oysters with a description of a new genus, *Mikrocytos* (g.n.), and two new species, *Mikrocytos mackini* (sp.n.) and *Mikrocytos roughleyi* (sp.n.). Fish. Bull. 3, 581–593.

Gaffney, P.M., 1990. Enzyme heterozygosity, growth rate, and viability in *Mytilus edulis*: another look. Evolution 44, 204–210.

Gardner, N.C., Smith, M.S.R., Maguire, G.B., Kent, G., Nell, J.A., 1994. Studies on triploid oysters in Australia: III. Gametogenesis of diploid and triploid Pacific oysters, *Crassostrea gigas* (Thunberg), in Tasmania. pp. 178–193. In: Nell, J.A., Maguire, G.B. (Eds.), Evaluation of triploid Sydney rock oysters (*Saccostrea commercialis*) and Pacific oysters (*Crassostrea gigas*) on commercial leases in New South Wales and Tasmania, Final Report to FRDC, September 1994, NSW Fisheries, Port Stephens Research Centre, Taylors Beach, NSW and University of Tasmania, Launceston, Tas.

Gardner, C., Maguire, G.B., Kent, G.N., 1996. Studies on triploid oysters in Australia: VII. Assessment of two methods for determining triploidy: adductor muscle diameter, and nuclear size. J. Shellfish Res. 15, 609–615.

Gendreau, S., Grizel, H., 1990. Induced triploidy and tetraploidy in the European flat oyster, *Ostrea edulis* L. Aquaculture 90, 229–238.

Gérard, A., Peignon, J.M., Chagot, D., 1991. Contrôle de la ploïdie par imagerie numérique dans des expériences d'induction de la triploïdie chez les mollusques bivalves, Congrés CIEM La Rochelle. PAPER ICES C.M. 1991/F:12 Réf. K, Mariculture Committee, 6 pp.

Gérard, A., Naciri, Y., Peignon, J.M., Ledu, C., Phelipot, P., Noiret, C., Peudenire, I., Grizel, H., 1994a. Image analysis: a new method for estimating triploidy in commercial bivalves. Aquacult. Fish. Manage. 25, 697–708.

Gérard, A., Naciri, Y., Peignon, J.M., Ledu, C., Phelipot, P., 1994b. Optimization of triploid induction by the use of 6-DMAP for the oyster *Crassostrea gigas* (Thunberg). Aquacult. Fish. Manage. 25, 709–719.

Goulletquer, P., Joly, J.P., Gérard, A., Le Gagneur, E., Moriceau, J., Peignon, J.M., Heurtebise, S., Phelipot, P., 1995. Performance of triploid Pacific oysters *Crassostrea gigas* (Thunberg) reared in high carrying capacity ecosystem: survival, growth and proximate biochemical composition. Haliotis 25, 1–12.

Guo, X., Allen Jr., S.K., 1994a. Reproductive potential and genetics of triploid Pacific oysters, *Crassostrea gigas* (Thunberg). Biol. Bull. 187, 309–318.

Guo, X., Allen Jr., S.K., 1994b. Viable tetraploids in the Pacific oyster (*Crassostrea gigas* Thunberg) produced by inhibiting polar body 1 in eggs from triploids. Mol. Mar. Biol. Biotechnol. 3 (1), 42–50.

Guo, X., Allen Jr., S.K., 1994c. Sex determination and polyploid gigantism in the dwarf surfclam (*Mulina lateralis* Say). Genetics 138, 1199–1206.

Guo, X., Cooper, K., Hershberger, W.K., Chew, K.K., 1992. Genetic consequences of blocking polar body I with cytochalasin B in fertilized eggs of the Pacific oyster, *Crassostrea gigas*: I. Ploidy of resultant embryos. Biol. Bull. 183, 381–386.

Guo, X., Hershberger, W.K., Cooper, K., Chew, K.K., 1994. Tetraploid induction with mitosis I inhibition and cell fusion in the Pacific oyster (*Crassostrea gigas* Thunberg). J. Shellfish Res. 13, 193–198.

Guo, X., DeBrosse, G.A., Allen Jr., S.K., 1996. All-triploid Pacific oysters (*Crassostrea gigas* Thunberg) produced by mating tetraploids and diploids. Aquaculture 142, 149–161.

Hand, R.E., Nell, J.A., 1999. Studies on triploid oysters in Australia: XII. Gonad discolouration and meat condition of diploid and triploid Sydney rock oysters (*Saccostrea commercialis*) in five estuaries in New South Wales, Australia. Aquaculture 171, 181–194.

Hand, R.E., Nell, J.A., Maguire, G.B., 1998a. Studies on triploid oysters in Australia: X. Growth and mortality of diploid and triploid Sydney rock oysters *Saccostrea commercialis* (Iredale and Roughley). J. Shellfish Res. 17, 1115–1127.

Hand, R.E., Nell, J.A., Smith, I.R., Maguire, G.B., 1998b. Studies on triploid oysters in Australia: XI. Survival of diploid and triploid Sydney rock oysters, *Saccostrea commercialis* (Iredale and Roughley) through outbreaks of winter mortality caused by *Mikrocytos roughleyi* infestation. J. Shellfish Res. 17, 1129–1135.

Hand, R.E., Nell, J.A., Reid, D.D., Smith, I.R., Maguire, G.B., 1999. Studies on triploid oysters in Australia: effect of initial size on growth of diploid and triploid Sydney rock oysters *Saccostrea commercialis* (Iredale and Roughley). Aquacult. Res. 30, 35–42.

Hawkins, A.J.S., Day, A.J., Gérard, A., Naciri, C., Ledu, C., Bayne, B.L., Héral, M., 1994. A genetic and metabolic basis for faster growth among triploids induced by blocking meiosis I but not meiosis II in the larviparous European flat oyster, *Ostrea edulis* L. J. Exp. Mar. Biol. Ecol. 184, 21–40.

He, M., Lin, Y., Shen, Q., Hu, J., Jiang, W., 2000. Production of tetraploid pearl oyster (*Pinctada martensii* Dunker) by inhibiting the first polar body in eggs from triploids. J. Shellfish Res. 19, 147–151.

Heasman, M., Goard, L., Diemar, J., Callinan, R.B., 2000. Improved early survival of molluscs, Aquaculture CRC Project A.2.1. NSW Fisheries Report Series: Report No. 29; NSW Fisheries, Port Stephens Fisheries Centre, Nelson Bay, NSW, 63 pp.

Jeong, B.Y., Ohshima, T., Koizumi, C., Kanou, Y., 1990. Lipid deterioration and its inhibition of Japanese oyster *Crassostrea gigas* during frozen storage. Nippon Suisan Gakkaishi 56, 2083–2091.

Jiang, W., Xu, G., Lin, Y., Li, G., 1991. Comparison of growth between triploid and diploid of *Pinctada martensii* (D.). Trop. Oceanol. Redai-Haiyang 10 (3), 1–7.

Komaru, A., Uchimura, Y., Ieyama, H., Wada, K.T., 1988. Detection of induced triploidy scallop, *Chlamys nobilis*, by DNA microfluorometry with DAPI staining. Aquaculture 69, 201–209.

Korac, S., Nell, J.A., Prescott, J., 1996. Studies on triploid oysters in Australia: VIII. Sensory evaluation of Sydney rock oysters *Saccostrea commercialis*. Asian Fish. Sci. 9, 61–68.

Langdon, C.J., Robinson, A.M., 1996. Aquaculture potential of the Suminoe oyster (*Crassostrea ariakensis* Fugita 1913). Aquaculture 144, 321–338.

Lee, K.H., Choe, W.K., Pyeun, J.H., Kim, M.N., 1976. Discolouration of canned, boiled oyster. Bull. Korean Fish. Soc. 9, 111–119.

Leitão, A., Boudry, P., Thiriot-Quiévreux, C., 2001. Negative correlation between aneuploidy and growth in the Pacific oyster, *Crassostrea gigas*: ten years of evidence. Aquaculture 193, 39–48.

Lindsay, C.E., Simons, D., 1997. The fisheries for Olympia oysters, *Ostreola conchaphila*; Pacific oysters, *Crassostrea gigas*; and Pacific razor clams, *Siliqua patula*, in the state of Washington. In: Mackenzie Jr., Cl., Burrell Jr., V.G., Rosenfield, A., Hobart, W.L. (Eds.), The History, Present Condition, and Future of the Molluscan Fisheries of North and Central America and Europe, Volume 2, Pacific Coast and Supplemental Topics. NOAA Technical Report NMFS 128, Scientific Publications Office, National Marine Fisheries Service, Seattle, WA, pp. 89–113.

Maguire, G.B., Boocock, B., Kent, G.N., Gardner, N.C., 1994a. Studies on triploid oysters in Australia: IV. Sensory evaluation of triploid and diploid Pacific oysters, *Crassostrea gigas* (Thunberg), in Tasmania. In: Nell, J.A., Maguire, G.B. (Eds.), Evaluation of triploid Sydney Rock Oysters (*Saccostrea commercialis*) and Pacific Oysters (*Crassostrea gigas*) on Commercial Leases in New South Wales and Tasmania, Final Report to FRDC, September, 1994, NSW Fisheries, Port Stephens Research Centre, Taylors Beach, NSW and University of Tasmania, Launceston, Tas., pp. 178–193.

Maguire, G.B., Gardner, N.C., Nell, J.A., Kent, G.N., Kent, A.S., 1994b. Studies on triploid oysters in Australia: II. Growth, condition index, glycogen content and gonad area of triploid and diploid Pacific oysters, *Crassostrea gigas* (Thunberg), in Tasmania. In: Nell, J.A., Maguire, G.B. (Eds.), Evaluation of Triploid Sydney

Rock Oysters (*Saccostrea commercialis*) and Pacific Oysters (*Crassostrea gigas*) on Commercial Leases in New South Wales and Tasmania, Final Report to FRDC, September, 1994, NSW Fisheries, Port Stephens Research Centre, Taylors Beach, NSW and University of Tasmania, Launceston, Tas., pp. 37–71.

Mason, K.M., Shumway, S.E., Allen Jr., S.K., Hidu, H., 1988. Induced triploidy in the soft-shell clam *Mya arenaria*: energetic implications. Mar. Biol. 98, 519–528.

Matthiessen, G.C., Davis, J.P., 1992. Observations on growth rate and resistance to MSX (*Haplosporidium nelsoni*) among diploid and triploid eastern oysters (*Crassostrea virginica* (Gmelin, 1791)) in New England. J. Shellfish Res. 11, 449–454.

Meyers, J.A., Burreson, E.M., Barber, B.J., Mann, R., 1991. Susceptibility of diploid and triploid Pacific oysters, *Crassostrea gigas* (Thunberg, 1793) and eastern oysters, *Crassostrea virginica* (Gmelin, 1791), to *Perkinsus marinus*. J. Shellfish Res. 10, 433–437.

Moore, C.A., Gelder, S.R., 1987. Receptor sites in hemocyte recognition. Am. Zool. 27, 98A.

Nell, J.A., 1993. Farming the Sydney rock oyster (*Saccostrea commercialis*) in Australia. Rev. Fish. Sci. 1, 97–120.

Nell, J.A., Maguire, G.B., 1998. Commercialisation of triploid Sydney rock and Pacific oysters: Part 1. Sydney rock oysters. Final Report to Fisheries Research and Development Corporation (FRDC), NSW Fisheries Final Report Series, Report No. 10, NSW Fisheries, Port Stephens Research Centre, Taylors Beach, NSW, 121 pp.

Nell, J.A., Cox, E., Smith, I.R., Maguire, G.B., 1994. Studies in triploid oysters in Australia: I. The farming potential of triploid Sydney rock oysters *Saccostrea commercialis* (Iredale and Roughley). Aquaculture 126, 243–255.

Nell, J.A., Hand, R.E., Goard, L.J., McAdam, S.P., Maguire, G.B., 1996. Studies on triploid oysters in Australia: evaluation of cytochalasin B and 6-dimethylaminopurine for triploidy induction in Sydney rock oysters *Saccostrea commercialis* (Iredale and Roughley). Aquacult. Res. 27, 689–698.

Nell, J.A., McMahon, G.E., Hand, R.E., 1998. Tetraploidy induction in Sydney rock oysters. Aquaculture CRC Project D.4.2, NSW Fisheries Final Report Series, Report No. 9, NSW Fisheries, Port Stephens Research Centre, Taylors Beach, NSW, 25 pp.

Perkins, F.A., Wolf, P.H., 1976. Fine structure of *Marteilia sydneyi* sp. n.—haplosporidian pathogen of Australian oysters. J. Parasitol. 62, 528–538.

Quillet, E., Panelay, P.J., 1986. Triploidy induction by thermal shocks in the Japanese oyster, *Crassostrea gigas*. Aquaculture 57, 271–279.

Sami, S., Shpigel, M., Faisal, M., 1991. Comparison between the host defense mechanism of diploid and triploid oysters *Crassostrea gigas*: effect of thermal stress on hemocyte surface Concanavalin A-binding sites. Z. Angew. Zool. 78, 69–78.

Scarpa, J., Toro, J.E., Wada, K.T., 1994. Direct comparison of six methods to induce triploidy in bivalves. Aquaculture 119, 119–133.

Shen, Y.P., Zhang, X.Y., He, H.P., Ma, L.J., 1993. Triploidy induction by hydrostatic pressure in the pearl oyster, *Pinctada martensii* Dunker. Aquaculture 110, 221–227.

Shpigel, M., Barber, B.J., Mann, R., 1992. Effects of elevated temperature on growth, gametogenesis, physiology, and biochemical composition in diploid and triploid Pacific oysters, *Crassostrea gigas* Thunberg. J. Exp. Mar. Biol. Ecol. 161, 15–25.

Stanley, J.G., Allen Jr., S.K., Hidu, H., 1981. Polyploidy induced in the American oyster, *Crassostrea virginica*, with cytochalasin B. Aquaculture 23, 1–10.

Stanley, J.G., Hidu, H., Allen Jr., S.K., 1984. Growth of American oysters increased by polyploidy induced by blocking meiosis I but not meiosis II. Aquaculture 37, 147–155.

Supan, J.E., 2000. Tetraploid eastern oysters: an arduous effort. J. Shellfish Res. 19, 655 (abstract).

Supan, J.E., Wilson, C.E., Allen Jr., S.K., 2000. The effect of cytochalasin B dosage on the survival and ploidy of *Crassostrea virginica* (Gmelin) larvae. J. Shellfish Res. 19, 125–128.

Tian, C., Wang, R., Liang, Y., Wang, Z., Yu, R., 1999. Triploidy of *Crassostrea gigas* induced with 6-DMAP: by blocking of the second polar body of the zygotes. J. Fish. Sci. China 6 (2), 1–4.

Thiriot-Quievréux, C., Noël, T., Bougrier, S., Dallot, S., 1988. Relationships between aneuploidy and growth rate in pair matings of the oysters *Crassostrea gigas*. Aquaculture 75, 89–96.

Thiriot-Quievréux, C., Pogson, G.H., Zouros, E., 1992. Genetics of growth rate variation in bivalves: aneuploidy and heterozygosity effects in a *Crassostrea gigas* family. Genome 35, 39–45.

Uchimura, Y., Komaru, A., Wada, K.T., Ieyama, H., Yamaki, M., Furuta, H., 1989. Detection of induced triploidy at different ages for larvae of the Japanese pearl oyster, *Pinctada fucata martensii*, by microfluorometry with DAPI staining. Aquaculture 76, 1–9.

Utting, S.D., Child, A., 1994. Genetic manipulation of the Manila clam (*Tapes philippinarum*) using cytochalasin B to induce triploidy. Aquaculture 44, 133–144.

Wang, Z., Guo, X., Allen Jr., S.K., Wang, R., 1999. Aneuploid Pacific oyster (*Crassostrea gigas* Thunberg) as incidentals from triploid production. Aquaculture 173, 347–357.

Wang, Z., Guo, X., Allen Jr., S.K., Wang, R., 2001. Heterozygosity and body size in triploid Pacific oysters, *Crassostrea gigas* Thunberg, produced from Meiosis II inhibition and tetraploids. Aquaculture (in press).

Yamamoto, S., Sugawara, Y., Nomura, T., Oshino, A., 1988. Induced triploidy in the Pacific oyster *Crassostrea gigas*, and performance of triploid larvae. Tohoku J. Agric. Res. 39 (1), 47–59.

Yamamoto, S., Sugawara, Y., Nomura, T., 1990. Chemical and thermal control of triploid production in Pacific oysters and mussels, with regard to controlling meiotic maturation. In: Hoshi, M., Yamashita, O. (Eds.), Adv. Invertebr. Reprod., vol. 5. Elsevier, Amsterdam, pp. 455–460.

Zaroogian, G., Yevich, P., 1993. Cytology and biochemistry of brown cells in *Crassostrea virginica* collected at clean and contaminated stations. Environ. Pollut. 79, 191–197.

Zouros, E., Thiriot-Quievréux, C., Kotoulas, G., 1996. The negative correlation between somatic aneuploidy and growth in the oyster *Crassostrea gigas* and implications for the effects of induced polyploidization. Genet. Res. Camb. 68, 109–116.