Project Completion Report

Creating a Tetraploid Broodstock for the Bay Scallop *Argopecten irradians*

Subaward # Z514702, Z520302, Z527901
Grant #2007-38500-18589, 2006-38500-17065, 2008-38500-19301

PROJECT CODE: 07-11 **SUBCONTRACT/ACCOUNT NO:** Z514702, Z520302, Z527901

PROJECT TITLE: Creating a Tetraploid Broodstock for the Bay Scallop *Argopecten irradians*

DATES OF WORK: July 1st 2008 to May 31, 2012.

PARTICIPANTS: : Rick Karney, Amandine Surier, Emma Green-Beach - Martha's Vineyard Shellfish Group, Inc.; Ximing Guo, Yongping Wang - Haskins Laboratory, Rutgers University ; Jack Blake - Sweet Neck Farm.

REASON FOR TERMINATION: The project was terminated when all the funds were expended.

PROJECT OBJECTIVES:
- Creating the first tetraploid broodstock for the bay scallop *Argopecten irradians*
- Producing the first natural all triploid bay scallop population that will meet FDA approval

ANTICIPATED BENEFITS:

This project seeked to improve the commercial feasibility of bay scallop aquaculture in the Northeast United States through the application of tetraploid technology.

Bay scallop farming in the US will be economically viable when growers have a bay scallop with an adductor muscle that reaches market size within a year and/or when overwintering survival is dramatically increased. The creation of a tetraploid broodstock to produce chemical free triploids was investigated in this study as a way to offset the high labor costs and the overwintering mortality issues.

PRINCIPAL ACCOMPLISHMENTS:

This NRAC project is a continuation of a one-year project funded by the Massachusetts Shellfish Aquaculture Innovation Consortium (MSAIC) though a grant from the Massachusetts Department of Agriculture Resources. Under the 2007 MSAIC grant, triploid groups F1 were produced in the bay scallop *Argopecten irradians* to establish the foundations for our 2008 NRAC funded experimental work.

Further accomplishments:
- The first worldwide tetraploid bay scallops were produced in August 2008 and survived as juveniles (3 mm) until overwintering.
- It was established that there is a clear visual difference between a ripe 2N scallop and a ripe 3N scallop. They can be separated to a certain extent by visual grading.
- Clear methodologies for tagging and sampling bay scallop for ploidy analysis were identified
- Conclusions were drawn on the potential of the tetraploid technology to change the bay scallop industry

IMPACTS: It was established that the scallop industry might benefit more from new marketing techniques (whole scallop market) or gear modification to improve winter mortalities rather than from the creation of a tetraploid broodstock.

RECOMMENDED FOLLOW-UP ACTIVITIES: No follow up activity is recommended.

SUPPORT:

YEAR	NRAC-USDA FUNDING	OTHER SUPPORT					TOTAL SUPPORT
		UNIVER-SITY	INDUSTRY	OTHER FEDERAL	OTHER	TOTAL	
2008/2009	$38,034		$2,500 (In-Kind)		$11,471 (In-Kind)	$13,971	$59,093
2009/2010	$43,044		$2,500 (In-Kind)		$11,843 (In-Kind)	$14,343	$59,964
2010/2011	$47,119		$2,500(In-Kind)		$12,236(In-Kind)	$14,736	$61,855
TOTAL	$128,197		$7,500 (In-Kind)		$35,550 (In-Kind)	$43,050	$180,912

Other: Martha's Vineyard Shellfish Group, Inc

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED:

Papers Presented

Surier A. et al., 2010. Creating a tetraploid broodstock for the bay scallop *Argopecten irradians*. Oral Presentation for the Milford Aquaculture meeting in Sheldon, CT, February 2010.

Surier A. et al., 2010. Creating a tetraploid broodstock for the bay scallop *Argopecten irradians*. Oral Presentation for the Aquaculture America and National Shellfisheries Association tri-annual meeting in San Diego, CA, March 2010.

Surier A. et al., 2011. Challenges in creating a tetraploid broodstock for the bay scallop *Argopecten irradians*. Oral Presentation for the Milford Aquaculture meeting in Sheldon, CT, February 2011.

Surier A. et al., 2011. Challenges in creating a tetraploid broodstock for the bay scallop *Argopecten irradians*. Oral Presentation for the National Shellfisheries Association meeting in Baltimore, MD, March 2011.

Surier A. et al., 2012. Challenges in creating a tetraploid broodstock for the bay scallop *Argopecten irradians*. Oral Presentation for the Milford Aquaculture meeting in Sheldon, CT, February 2012.

Surier A. et al., 2012. Challenges in creating a tetraploid broodstock for the bay scallop *Argopecten irradians*. Oral Presentation for the National Shellfisheries Association meeting in Seattle, WA, March 2012.

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PART II

Previous findings (NRAC grant 2004): How does chemically induced triploidy affect bay scallops?

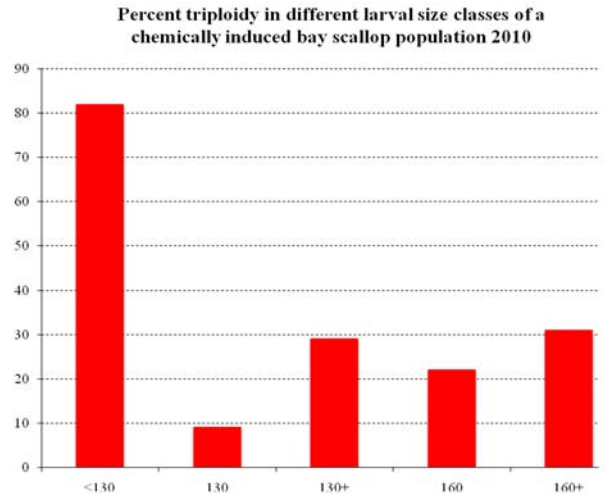
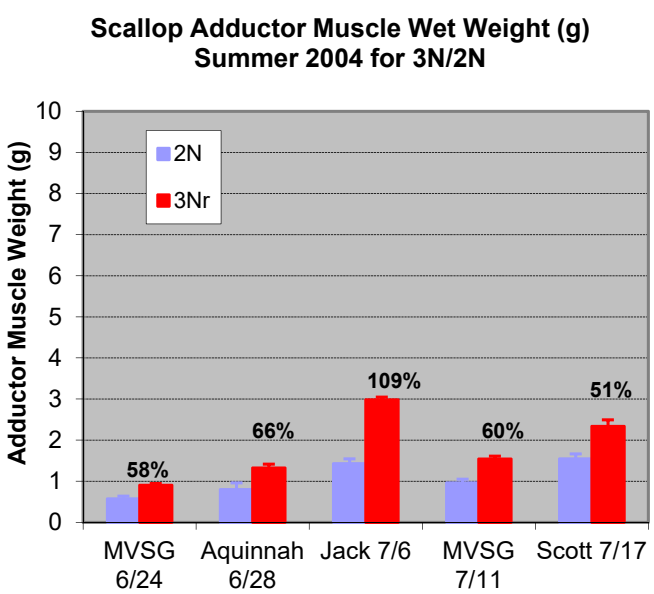


Figure 1. Percent triploidy in different larval size classes of a chemically induced bay scallop population, 2010.

- 3N scallop larvae underperform 2N larvae (figure 1)
- There is no significant increase in size (adductor muscle weight) the first season (triploidy does not create the one year scallop we hoped for to get around winter mortalities)
- However, adductor muscle is significantly larger the second season (energy used for sexual maturation is diverted towards somatic growth).



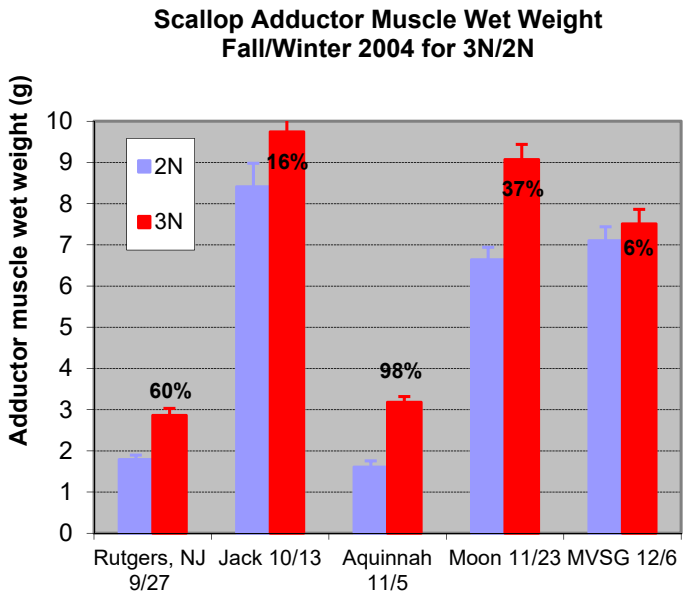


Figure 2 and 3: Scallop adductor muscle wet weight for triploid and diploid bay scallops for summer and fall/winter 2004.

The figure above shows that the mean adductor muscle wet weight in 3N was: 68.8 % greater in the summer (51% - 109%), 43.4% greater in the fall/winter (6% - 98%).

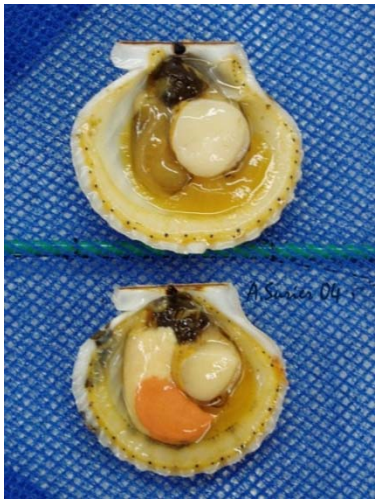


Figure 4: A second year triploid bay scallop (top) with underdeveloped gonad and large adductor muscle versus a regular diploid bay scallop (bottom) with developed gonad and small adductor muscle.

Conclusion: there is a significant advantage to triploidy for the bay scallops in the second year of growth.

- **Step 1 in producing the Tetraploid broodstock: producing the F1 triploid population**

  - Chemically induced 3N populations have been consistently produced every year between 2007 and 2011 and raised to juvenile size before overwintering.
  - Triploidy was induced with an 11 min 400 µM 6-DMAP treatment, inhibiting second polar body.
  - Success of induction varied between 0% and 97% (figure 5), the proportion of triploids in a population 3N can be increased by selecting the smaller scallops during the larval stage.

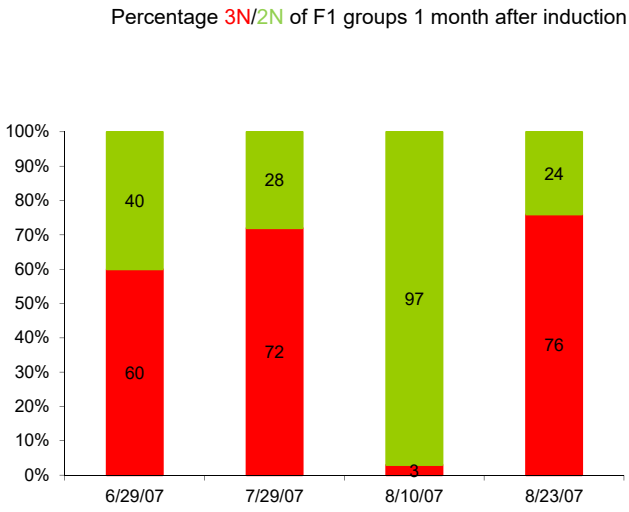


Figure 5 and 6: Inducing triploidy and raising the F1 triploid population

- The F1 populations were overwintered in bottom cages and pearl nets in Lagoon pond and Katama Bay.

Figure 7 and 8: Overwintering cages and pearl nets

➢ **Step 2 in producing the Tetraploid broodstock: Isolating a ripe triploid broodstock**

- Successful tagging and sampling protocols were identified (fig 9)
- Gill samples were easy to retrieve, provided a sufficient material for flow cytometry analysis and did not cause any mortality. (figure 10)

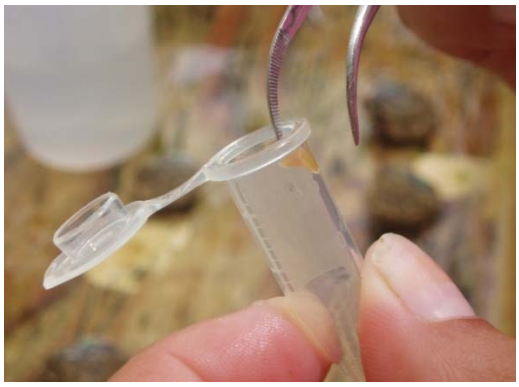


Figure 9 and 10: Tagging and sampling gills.

The triploid F1 broodstock was monitored for gonad development in the late spring early summer. Monitoring revealed the presence of sperm early on which disappeared later in the season. Only 5% of the triploids showed signs of eggs in August. This could mean that Triploidy induces protandry in the hermaphrodite *Argopecten irradiants.*

Figure 11: Early spring slight sign of sperm Figure 12: Presence of eggs Figure 13: Presence of both eggs and sperm

➢ **Step 3 in producing the Tetraploid broodstock: Spawning 3N bay scallops**

Spawning triploid bay scallops was a worldwide first. We attempted spawns in the 2008 – 2009 – 2010 - 2011 summer seasons.

General observations:

- the 3N scallops spawned easily the first year (2008) (figure 14)
- 3N scallops spawned mostly as female
- a few also spawned as males
- spawning window is shorter for 3N

Total 3N egg yield:

- 2008 - 20 million eggs (3 spawns) (figure 15)
- 2009 – 4 million eggs (4 spawns) (no survival)
- 2010 and 2011– did not spawn (2 attempts)

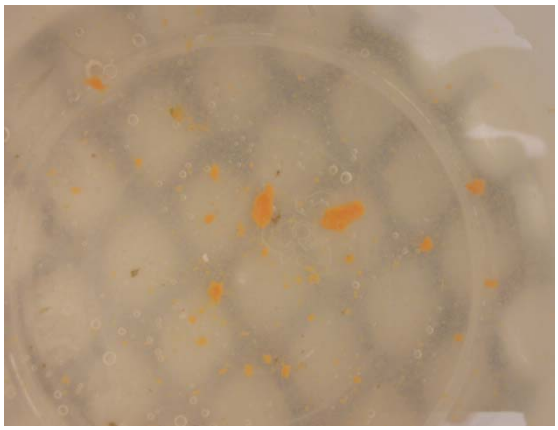


Figure 14 and 15: A Triploid scallop spawning eggs and triploid scallop eggs in a beaker

➢ **Step 4: Inducing tetraploidy in fertilized triploid eggs**

Tetraploidy was induced with an 11 min 400 µM 6-DMAP treatment, inhibiting first polar body in triploid eggs fertilized with diploid sperm. Success of treatment is shown in graph below:

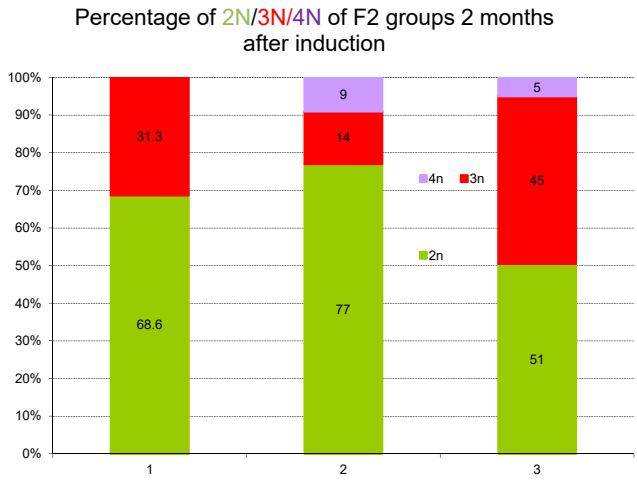


Figure 16 and 17: Proportion of 2N 3N and 4N in the F2 population in 2008/ treating the eggs with chemicals

At 2 months, flow cytometry analysis revealed the presence of tetraploids in two of the three groups produced (figure 16).

- **Step 5: Growing the tetraploid mix groups F2**

  - larvae were unusually orange (figure 18)
  - initial mortality was high
  - at first draindown we observed 89% abnormal larvae (strange blastulae, one shelled)
  - after 7 days we observed 97% mortality (in Oysters 95.6%, *Eudeline et al, 2000*)

The F2 4N mix groups were grown in down welling recirculating systems until late October (figure 19). They were deployed when they reached a size larger than 3mm in Katama Bay and overwintered in bottom cages along with the 3N F1 survivors (figure 20).

Figure 18: F2 larvae at drain down

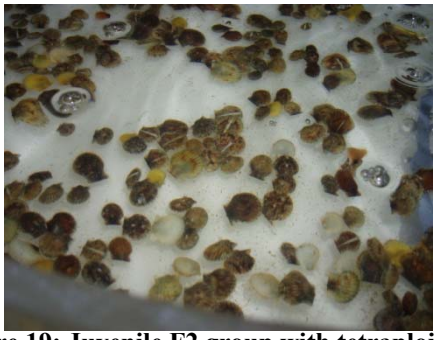


Figure 19: Juvenile F2 group with tetraploids

Figure 20: overwintering cages

- **Step 5 - Isolating the 4N (only took place in 2009)**

In April, survivors F2 4N/3N/2N mix were pulled out cages and transferred to raceways.
F2: high mortality, fouled with barnacles and mussel seed

F2 mix was gently cleaned and put in lanterns to grow large enough to be sampled

Late June, tagging and sampling began on the F2 mixed population to isolate the tetraploids: during the 2009 season, testing of 426 scallops from F2 mixed population isolated 2N and 3N but no 4N.

The tetraploids did not survive overwintering. Further attempts to produce the tetraploids in consecutive years were unsuccessful.

- **Results and General Challenges:**

- The induced triploids do not perform as well as the regular diploids the first growth year (fragile larvae/juveniles)
- The ripe triploids used for the F2 broodstock are difficult to identify and only a few will produce significant amounts of eggs
- The chemical treatment used to induce tetraploidy results in extremely high mortality
- We were able to produce a tetraploid bay scallop but they died after the juvenile stage and we were never able replicate our 2008 results

So how is the tetraploid technology so widely used with pacific oysters on the West Coast and in France? Scallops present different challenges than oysters: they are hermaphrodite, short lived, they have to be spawn naturally (stripping is not an option), they are overall more sensitive and the spawning

window is shorter. The only advantage in using scallops is that sampling for tissue is easier; there is no need for drilling through the shell.

- **Conclusions**

- Tetraploid bay scallops are biologically possible to produce but the cost for producing such broodstock is extremely high and lengthy (the process would take at least 3 years from triploids F1 to adult 4N)
- The broodstock, if produced, would likely not survive beyond the 2nd year
- The loss of genetic diversity found in hatchery seed, and especially in triploid hatchery seed increases the vulnerability of growers crops to diseases
- The scallop industry might benefit more from new marketing techniques (whole scallop market) or gear modification to improve winter mortalities rather than from the creation of a tetraploid broodstock.