

# Potential Bivalve Reservoirs of *Bonamia* spp. in Florida's Indian River Lagoon

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**HARBOR BRANCH**

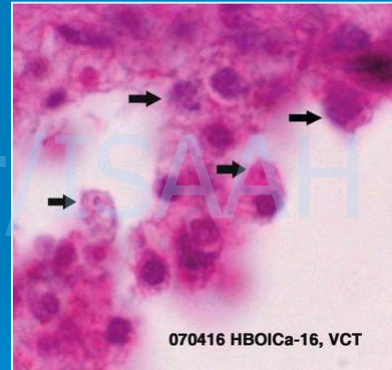
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## Background

- **Oyster mesocosm experiments in 2007**
  - Eastern oyster (*Crassostrea virginica*) and suminoe oyster (*C. ariakensis*)
  - *Bonamia* spp. positive by PCR
    - *C. ariakensis* also positive histologically
    - First report of *Bonamia* sp. in Florida waters
    - *B. exitiosa* (VIMS)
  - Similar *Bonamia* sp. caused mortalities in *C. ariakensis* in North Carolina (Audemard et al, 2008 )
  - Other *Bonamia* sp. reported in NC
    - *B. perspora* in *Ostreola equestris* (Carnegie et al, 2005)

## Background Information

- **What is *Bonamia*?**
  - haplosporidian parasite
- **Tissues infected**
  - haemocytes
- **Transmission**
  - water
- **Environmental associations**
  - warm temperature (20-32°C)
  - high salinity (30-35 ppt)



## *Bonamia* species

- ***B. ostrea* (Europe, West coast USA)**
  - *Ostrea edulis*, *O. angasi*, *O. chilensis*, *O. puelchana*,  
*O. denselamellosa*, *Crassostrea ariakensis*, *C. angulata*
- ***B. exitiosa* (New Zealand, Chile)**
  - *O. chilensis*, *O. angasi*
- ***B. roughleyi* (Australia)**
  - *Saccostrea glomerata*
- ***B. perspora* (U.S.)**
  - *Ostreola equestris*

## Objectives

- **Examine a range of bivalve species in the Indian River Lagoon, FL for *Bonamia***
  - Identify *Bonamia* spp.
  - Determine range of *Bonamia* spp.
    - Environmental component
- **Test collected bivalves for the presence of other pathogens**
  - *Perkinsus marinus*, *Haplosporidium nelsoni*



## Materials and Methods

- **Study site locations**
  - Southern portion of the Indian River Lagoon
    - 10 sites
- **Field collection**
  - 10-20 bivalves per species per location
- **Sample processing**
  - Measurements
  - Taxonomic identification
  - Tissue processing
    - PCR
    - Histology



# Indian River Lagoon



- Nation's most diverse estuary
  - 2200 animal species
  - 2100 plant species
- 156 miles long
- 5 counties
- 4 inlets

Study area



## Collection

- **GPS Coordinates**
- **Water quality data**
  - temperature
  - salinity
  - dissolved oxygen
- **Intertidal and subtidal regions**
  - oyster reefs/clumps
  - base of mangrove roots
  - wooden pilings
  - near inlets, discharge sites, intermediate sites
- **10-20 individuals per species**



## Sample Processing

- **Measurements**
  - shell height (mm)
  - weight (g)
- **DNA extraction**
  - Gill tissue
  - Roche High Pure PCR Template Preparation Kit
- **PCR**
  - Primers *Bonamia*:
    - Bon CF/CR, *Bonamia* spp. (300 bp)
    - CaBon146F/461R, *B. exitiosa* (316 bp)
  - *Perkinsus marinus*:
    - Pm F/R (307 bp)
  - *Haplosporidium nelsoni*:
    - MSX A/B (564 bp)
- **Histology**
  - Cross sections fixed in Davidson's
    - Transferred to 70% ethanol

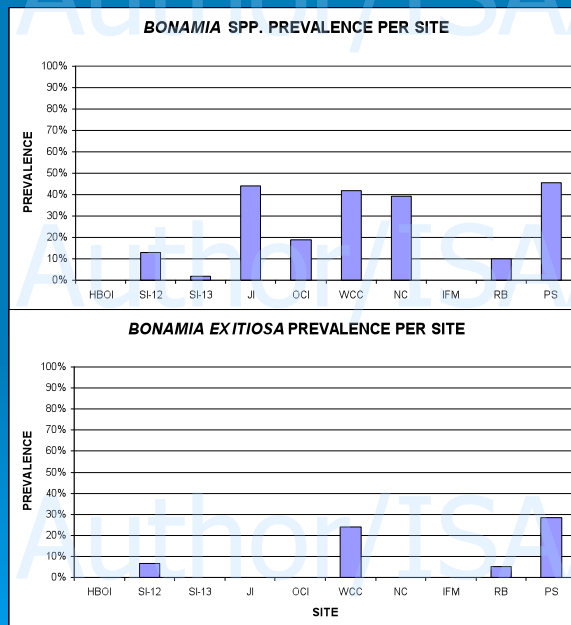


# Results

## Total Number Collected

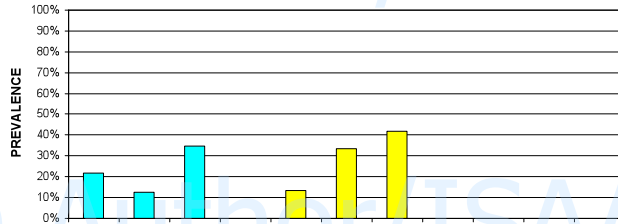
Species	HBOI	SI-12	SI-13	Jl	OCI	WCC	NC	IFM	RB	PS
<i>C. virginica</i>	10	10	8	10	10	10	15	12	15	15
<i>O. equestris</i>	17	16	20	12	20	36	0	24	0	0
<i>I. alatus</i>	2	0	9	19	5	20	21	20	0	13
<i>B. citrinus</i>	3	0	0	0	0	0	0	0	0	0
<i>B. exustus</i>	12	16	17	3	0	20	1	12	0	0
<i>I. recurvum</i>	2	1	1	0	0	1	1	0	0	0
<i>G. granosissima</i>	5	0	2	6	0	15	17	5	5	7
<i>M. mercenaria</i>	0	2	0	0	0	1	0	0	0	0
<i>C. cancellata</i>	0	0	0	0	0	2	1	1	0	0
<i>P. pectinata</i>	0	0	0	0	0	0	0	6	0	0
<i>A. rigida</i>	0	1	0	0	2	0	0	0	0	0
<b>TOTAL PER SITE</b>	<b>51</b>	<b>46</b>	<b>57</b>	<b>50</b>	<b>37</b>	<b>105</b>	<b>56</b>	<b>80</b>	<b>20</b>	<b>35</b>

## Bonamia spp.

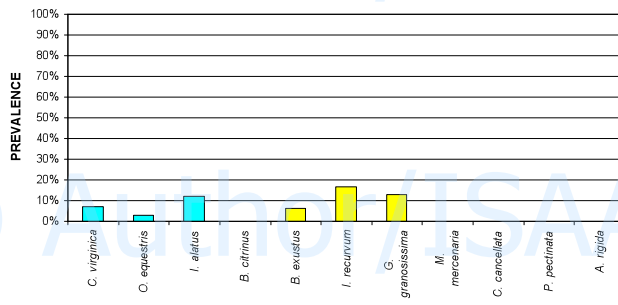


## Bonamia spp.

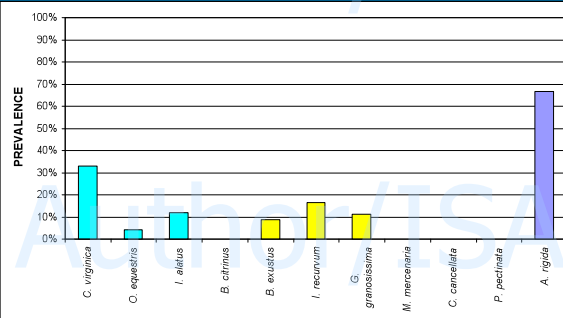
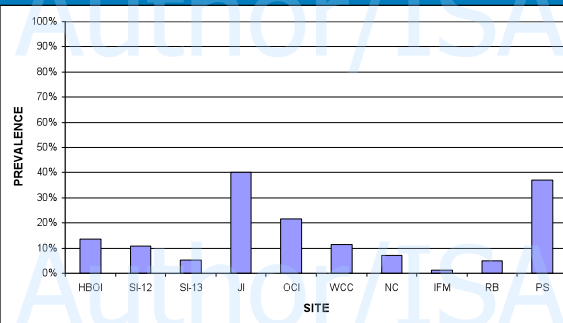
BONAMIA SPP. PREVALENCE PER SPECIES



BONAMIA EXITIOSA PREVALENCE PER SPECIES



## Perkinsus marinus



## Summary

### ➤ *Bonamia*

- 22% of all bivalves tested (n=537) were positive
  - 8/10 (80%) locations in the southern IRL
  - 6/11 (55%) species in the southern IRL
- Only 32.5% of *Bonamia* positives (n=120) were identified as *B. exitiosa*

### ➤ *Perkinsus marinus*

- 13% of all bivalves tested (n=537) were positive
  - 10/10 (100%) locations in the southern IRL
  - 7/11 (64%) species in the southern IRL

### ➤ *Haplosporidium nelsoni*

- 0% of all bivalves tested (n= 537) were positive

## Conclusions

- *Bonamia* spp. is present in multiple locations in the southern portion of the IRL
  - expands the known range of the parasite
- Multiple species of *Bonamia* spp. are present
  - *B. exitiosa*, *B. ostrea* (?), *B. perspora* (?)
- A wide variety of reservoir species exists for *Bonamia* spp.
- A wide variety of reservoir species exists for *Perkinsus marinus*
- *Haplosporidium nelsoni* was not detected



## Future Analysis

- **Histological confirmation of Bonamia PCR-positive samples**
  - Pathogenic?
- **Expansion of field collections to include a broader range of species and locations**
  - Lake Worth Lagoon (south of IRL)
  - Northern IRL, Gulf Coast, FL Keys
- **Further delineate role of the environment**
  - Salinity, temperature, disturbance

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Plate

