Visceral Toxicosis (Botulism) in Catfish

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Mission statement (mandate) of TCNWAC

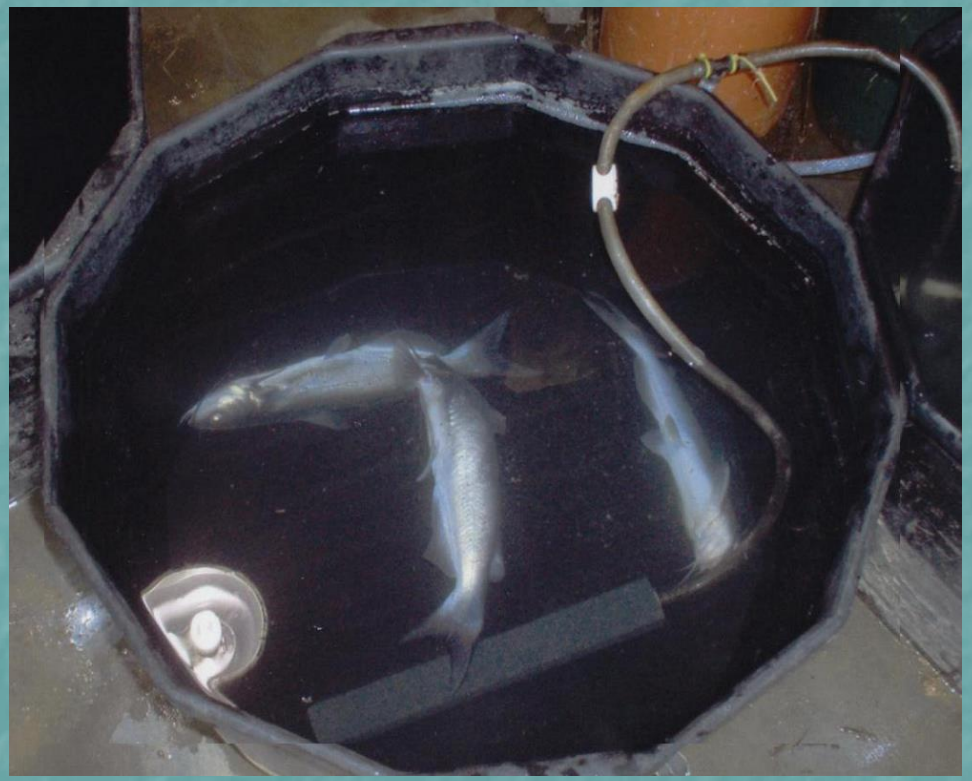
- Solve health problems of the catfish aquaculture industry through basic and applied research, extension, and diagnostic services
- Focus on the dissemination of research-based information to the catfish industry

Visceral toxicosis of catfish (VTC)

- Acute death of apparently healthy market and brooder size catfish
  - 25% pond mortality
- Occurs in late fall and early spring when pond temperatures are 18-22°C

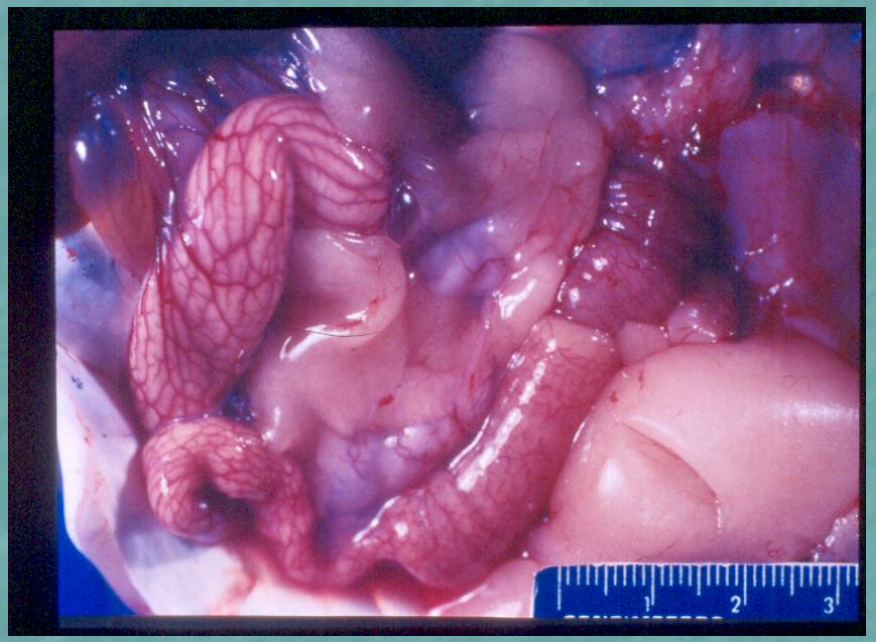
Clinical signs of VTC

- Abnormal schooling
- Jumping (“porpoising”) in ponds
- Terminally:
  - Erratic swimming along pond bank
  - Limp musculature



Lesions of VTC

- Externally: Portions of GI tract protruding from mouth
- Internally:
  - Chylous effusion
  - Congested spleen
  - Pale proximal intestine
  - Intussusceptions
  - Reticular pattern to liver



Botulinum (BoNT) as possible cause of VTC

Consistencies

- Neurologic signs
- Acute onset
- Seasonal
- Heated serum: not toxic

Inconsistencies

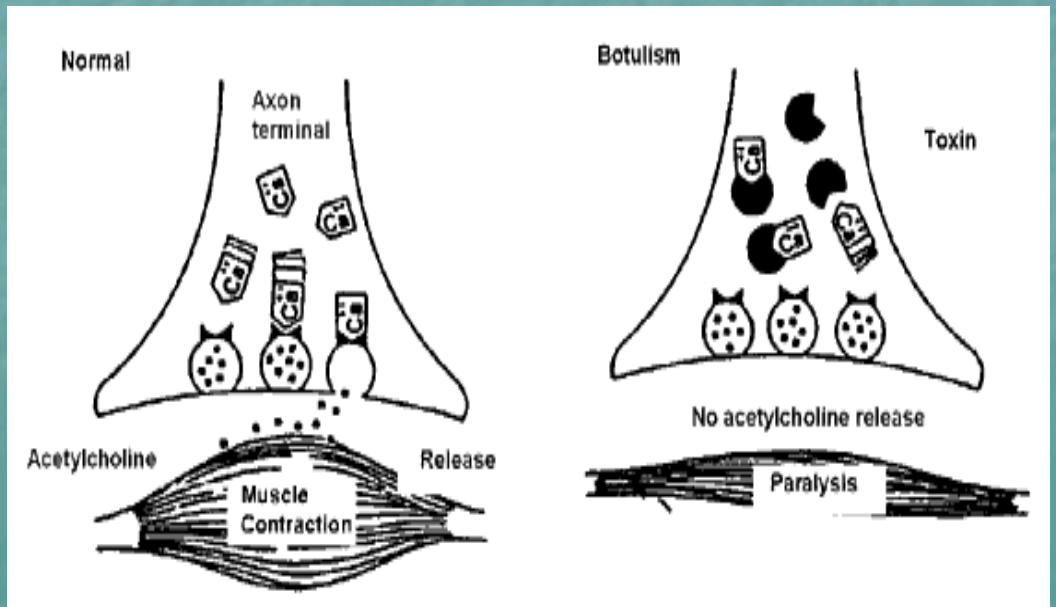
- Lesions
- Grain fed catfish carnivorous ?

Incriminated causes of VTC

- Infectious?
  - Negative bacterial cultures and virus isolations
- Toxic?
  - Blue green algae (G. Meerdink, U of Illinois)
    - HPLC assay negative for anatoxin, microcystin
    - Anatoxin a(s): Acetylcholinesterase normal
- Serum from VTC-affected fish injected into sentinel fish reproduced signs, lesions, mortality

Botulinum: Mechanism of Action

- Blocks release of acetylcholine from presynaptic neuron of neuromuscular junction
- Results in flaccid paralysis
- 7 serotypes of BoNT (A-G)



Preliminary diagnostics for botulism all negative

- Mouse bioassay (U Penn)
- Anaerobic cultures of intestinal contents (S. Zhang, MSU)
- PCR of liver, intestinal contents, kidneys, and spleen (P. Bowser, R. Getchell, Cornell U)
- Commercial rapid enzyme immunoassay kit (BADD)

Catfish neutralization assay

- Species of interest
- Catfish more sensitive to effects of BoNT than mouse?
- Fingerling catfish readily available for assay

Catfish neutralization assay methodology

- VTC serum incubated with each monospecific antitoxin: A, B, C, D, E, or F
  - 7 different VTC-affected sera tested
  - 3 fish each injected with VTC serum + monospecific antitoxin
- Positive control fish: VTC-affected sera
- Negative control fish: unaffected sera

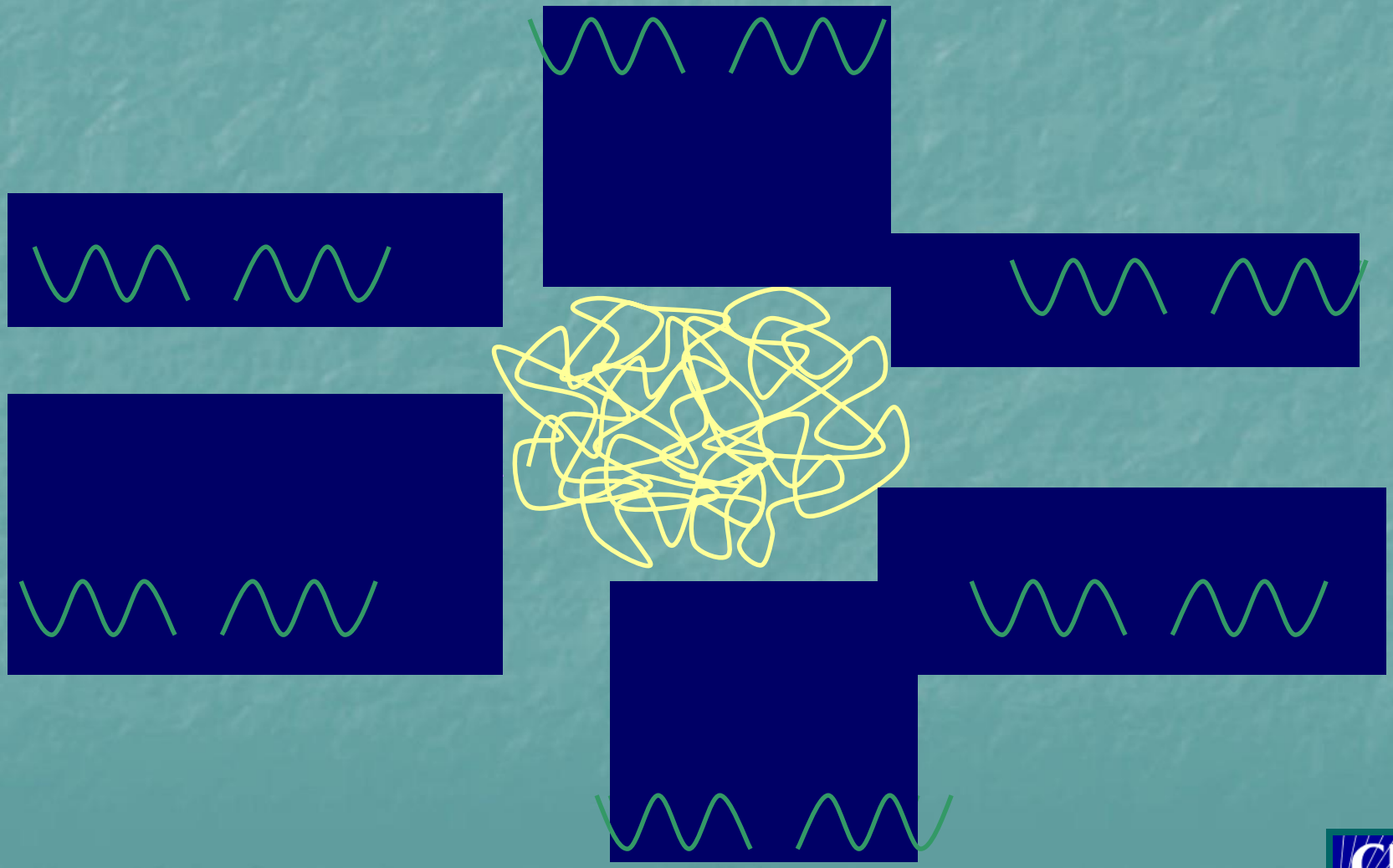
Catfish bioassay mortalities

Sample No.	Serum + A	Serum + B	Serum + C	Serum + D	Serum + E	Serum + F	Serum Only	Negative Control
1	3	3	-	-	0	-	3	0
2	3	3	-	-	0	-	2	0
3	3	3	-	-	0	-	3	0
4	3	3	-	-	0	0	3	0
5	3	1	-	-	0	0	3	0
6	2	0	-	-	0	0	1	0
7	2	1	-	-	0	-	3	0
4*	3	3	3	3	0	0	3	0

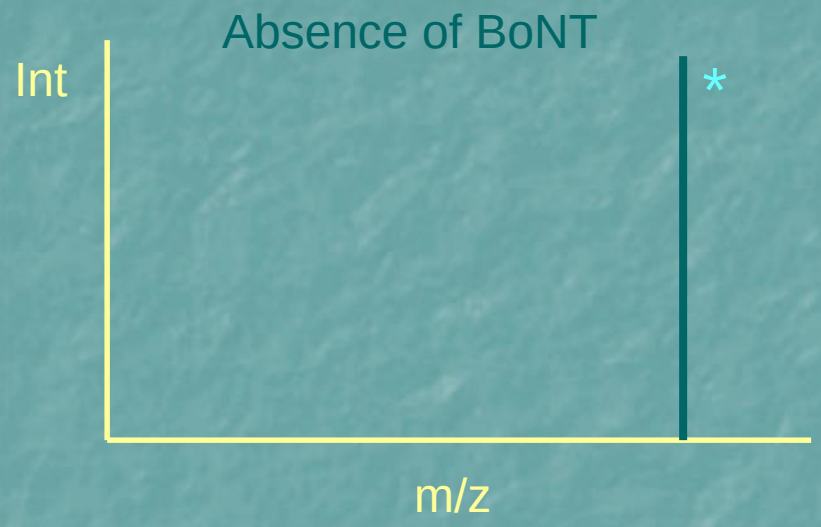
Additional diagnostic assay for BoNT: Endopep-MS (CDC)

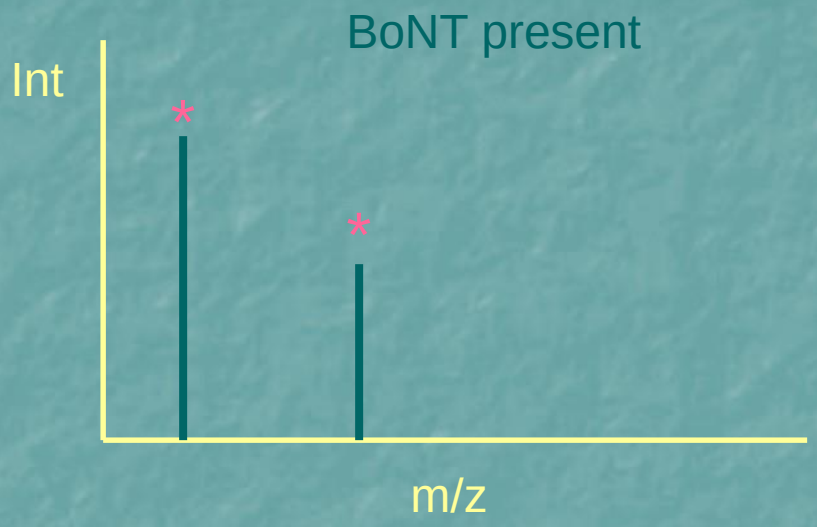
- BoNT + peptide substrate
  - Mimics natural *in vivo* target
  - Substrate cleaved in a specific, toxin-dependent location
- Cleavage products detected by mass spectrometry
  - Reports atomic weight of peptide cleavage products

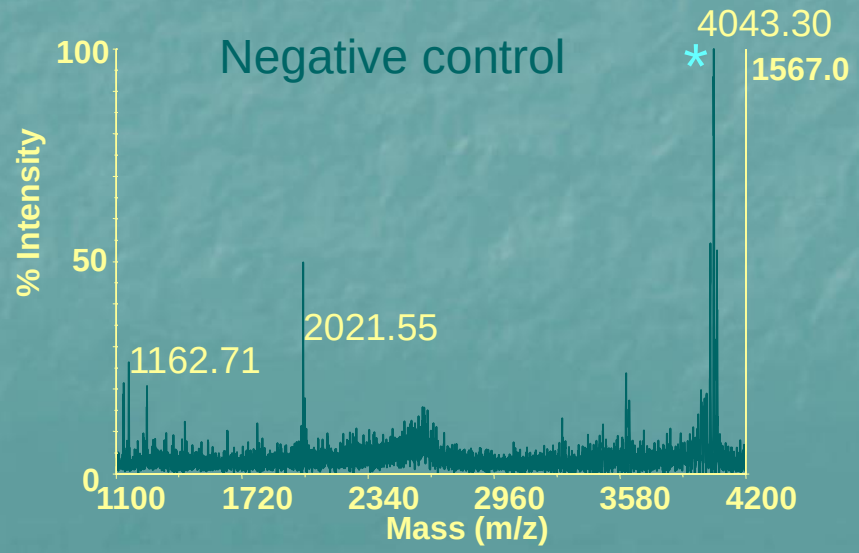
Endopep-MS Method

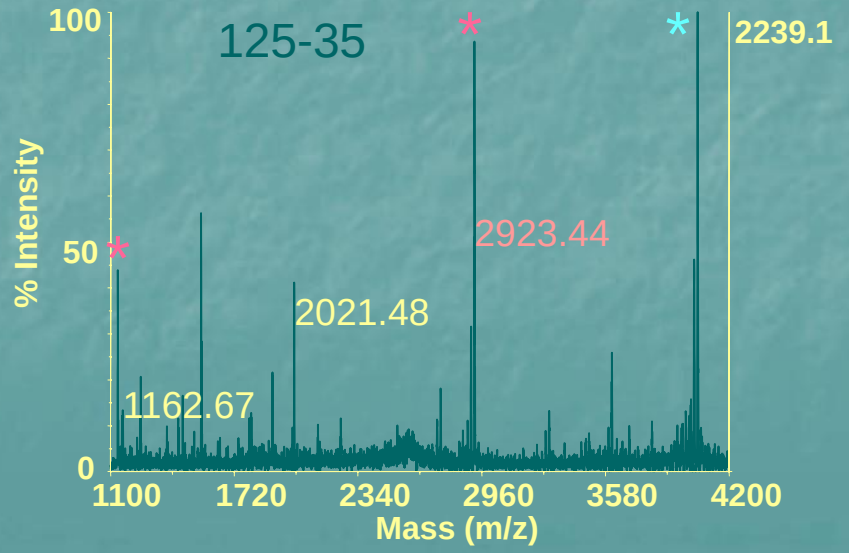


Detection of BoNT Activity by MS









Endopep MS results

- 6/6 VTC-affected samples positive for BoNT E
  - Estimated quantity: 0.01-0.5 LD50 MU/mL of serum
- 34/34 negative control catfish sera samples negative for BONT E

Implications of response actions taken: VTC management steps

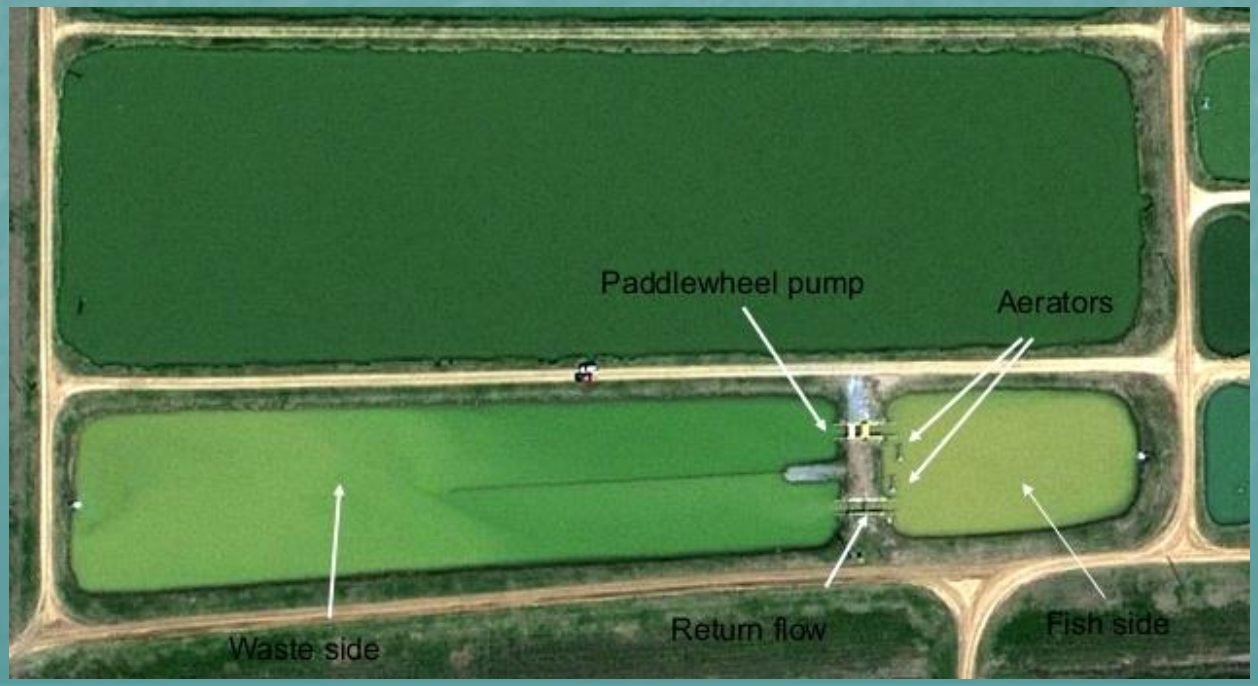
- Cost to industry estimated at $3.4 million annually

Immediate lessons learned

- Don’t overwinter large fish
- Feed large sized fish in winter
- Don’t feed temperature-sensitive live bait

Implications of response actions (cont)

- Use new technologies:
  - Split ponds
  - Intense aeration (10 horsepower/acre)
  - Vaccination for enteric septicemia of catfish (ESC)
  - Stock hybrid catfish-faster growth rate



Long term lessons learned about VTC

- Management of problem
  - Not always a cure or treatment for the problem, but can ameliorate its effects
- Catfish need to be fed during winter as temperatures fluctuate
- Avoid use of cold temperature sensitive live bait for brood fish during winter

Emergency preparations that need to be in place for VTC

- Remove carcasses from ponds (Easier in smaller ponds)
- Monitor pond temperatures daily during late fall and early spring
- Increase aeration in ponds
- Seek fish health professional help immediately with fish showing neurologic signs
- Harvest of fish from VTC-affected pond to spare pond without fish