

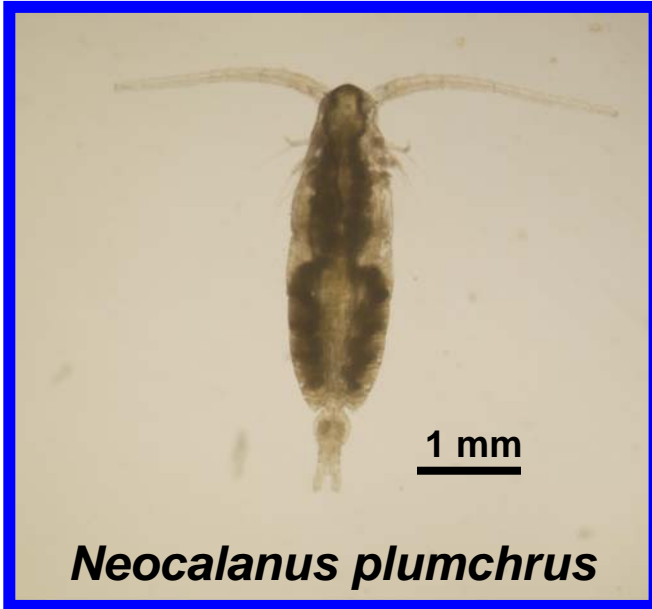


**A molecular method for species identification of
early life stage of
Neocalanus plumchrus and *Neocalanus flemingeri*
using Real-Time PCR**

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Atomospher Ocean Research Institute, Univ. of Tokyo**

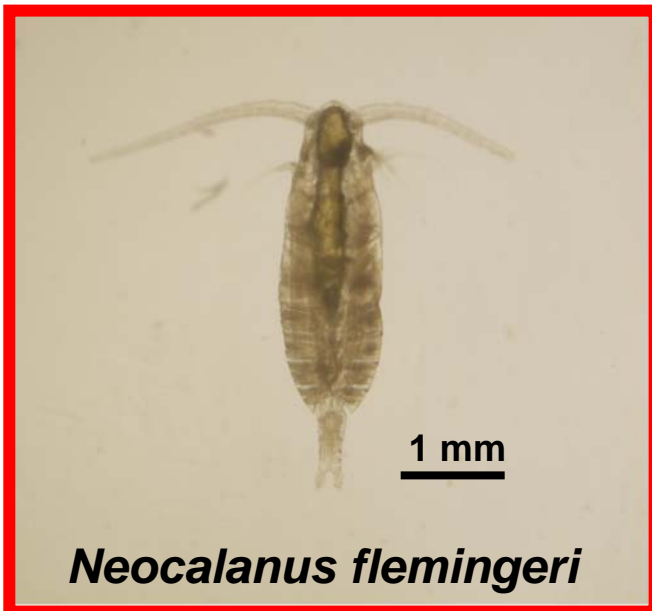


Neocalanus plumchrus, *Neocalanus flemingeri*



Neocalanus plumchrus

- North Pacific, Bering Sea
Sea of Japan and Sea of Okhotsk
- TL: 4.3 - 6.3 mm
- Dominate the mesozooplankton biomass through out the subarctic pacific ocean
(Mackas and Tsuda, 1999)

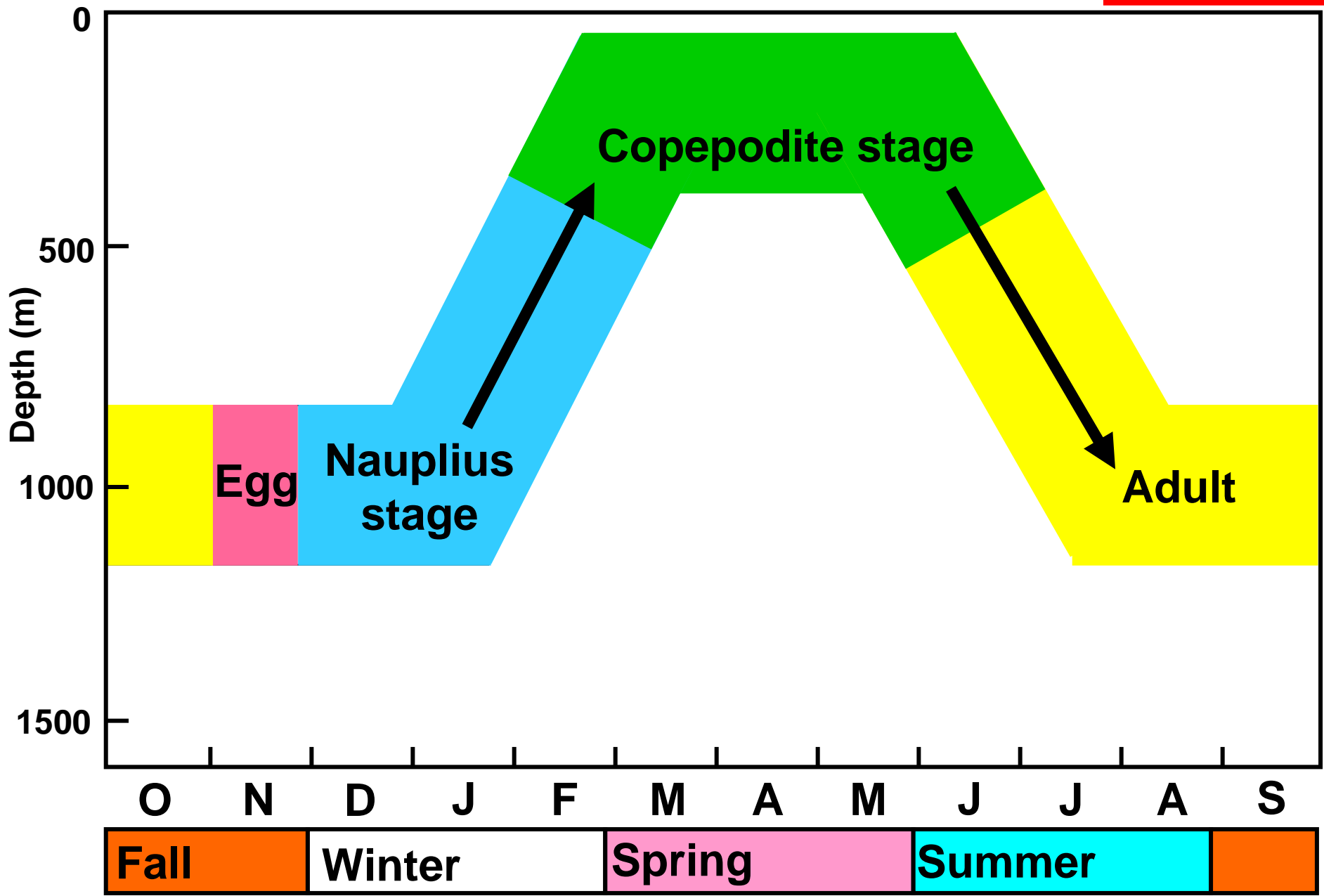


Neocalanus flemingeri

- Prey of high trophic level organisms
(Kobari et al. 2003)
- Transport large amount of carbon from surface to mesopelagic layer
(Kobari et al. 2008)

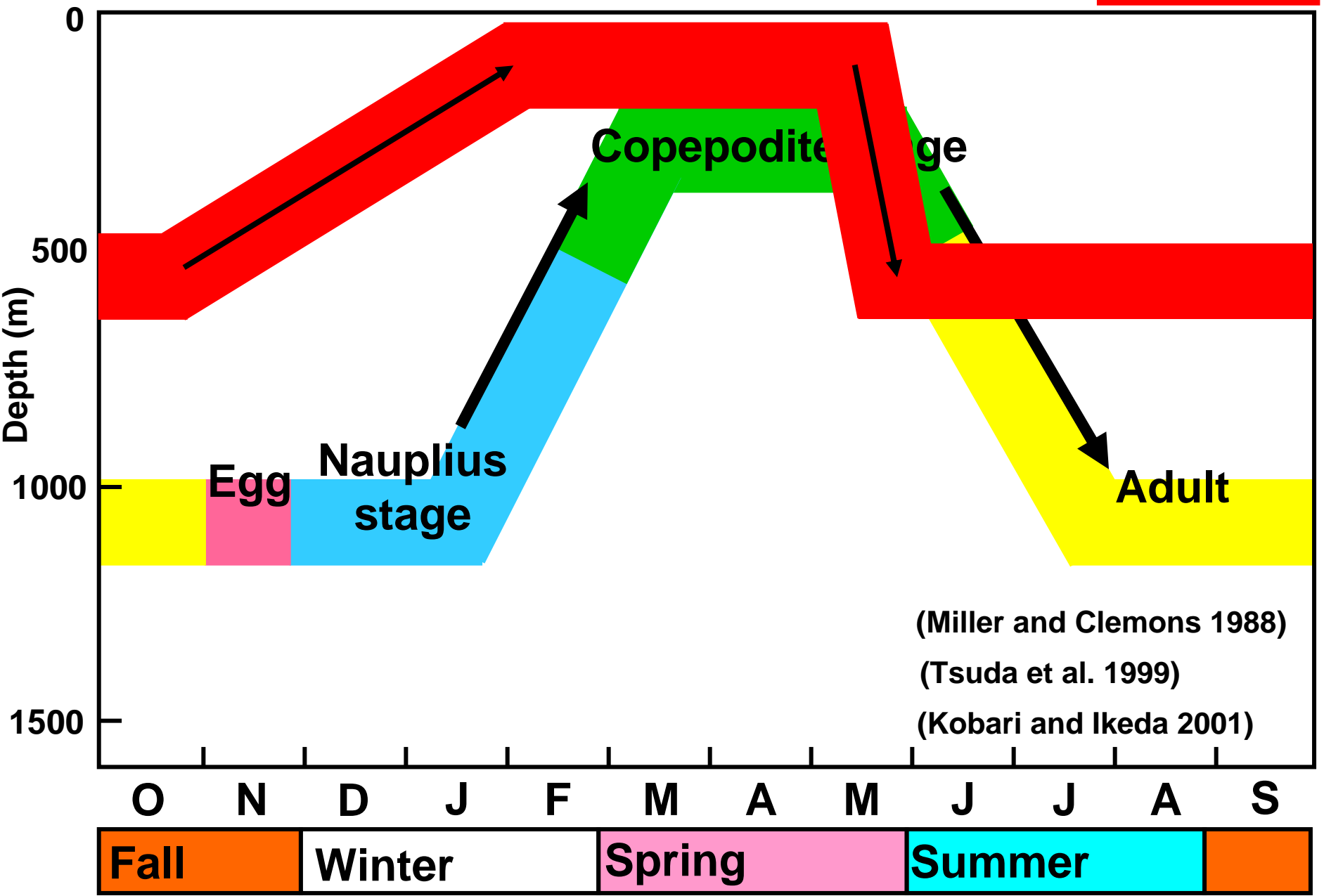
Life history of 2 species

N. plumchrus
N. flemingeri



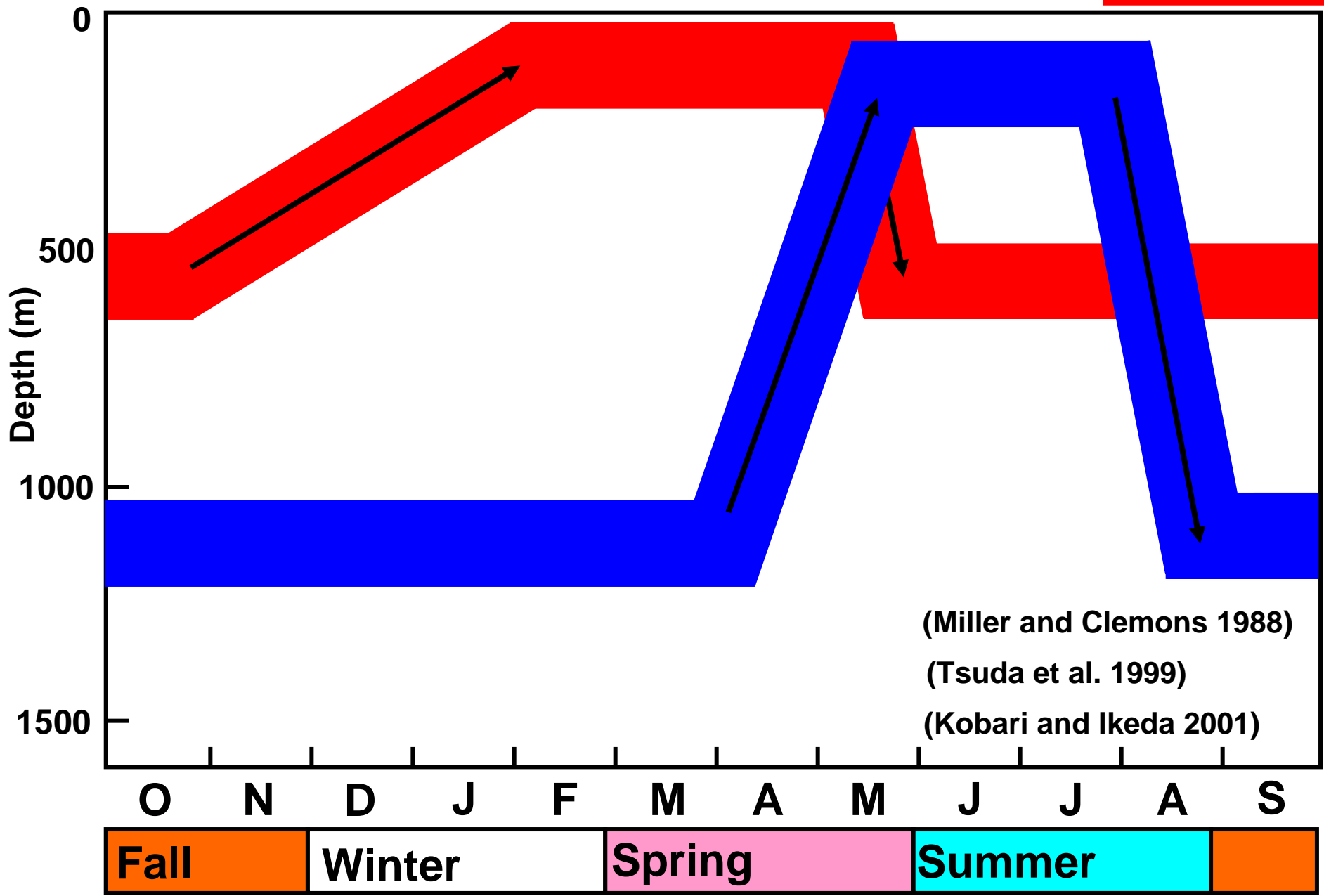
Life history of 2 species

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Life history of 2 species

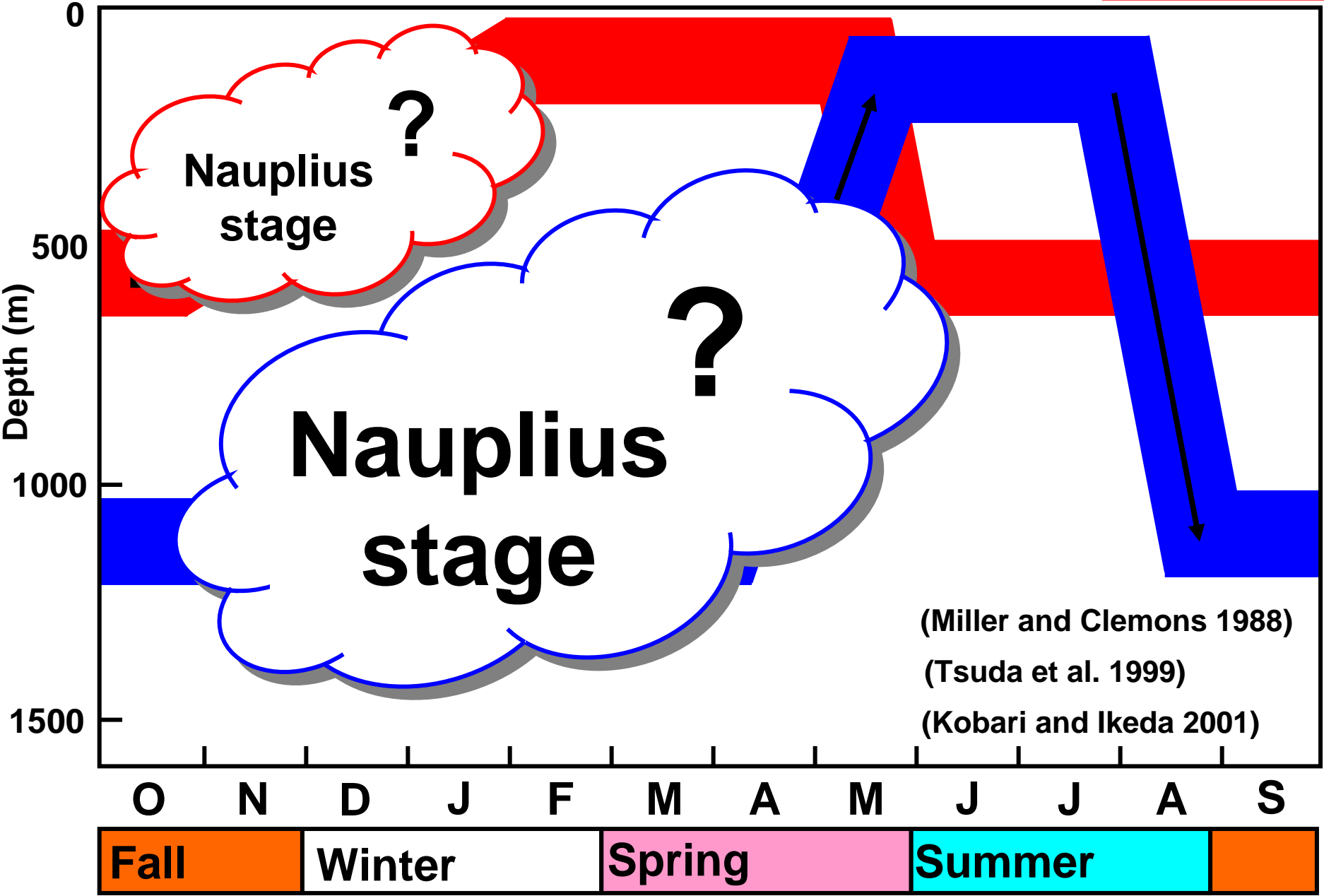
N. plumchrus
N. flemingeri



Life history of 2 species

N. plumchrus

N. flemingeri



Problems of nauplii investigation



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• Undeveloped morphological characteristic

⇒ Can be difficult or impossible to identify to species

We should develop a molecular method to identify

Problems of nauplii investigation



N. plumchrus



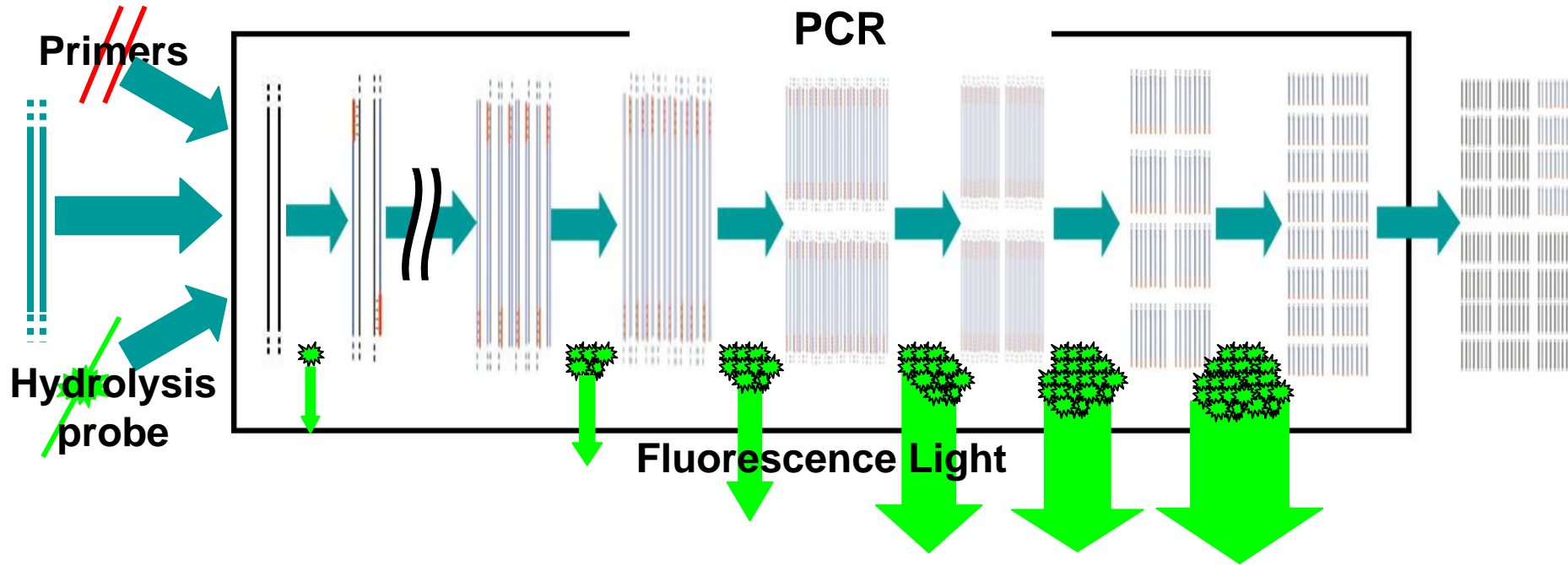
N. flemingeri

• Undeveloped morphological characteristic

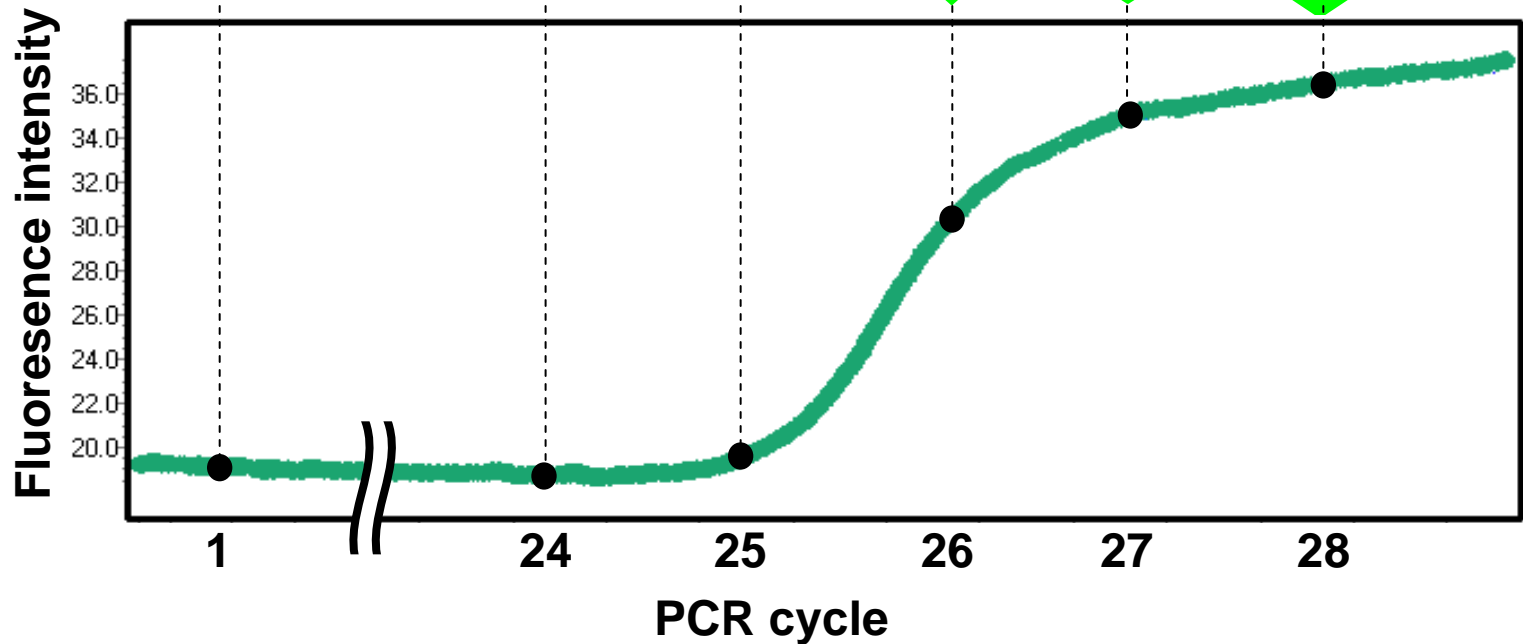
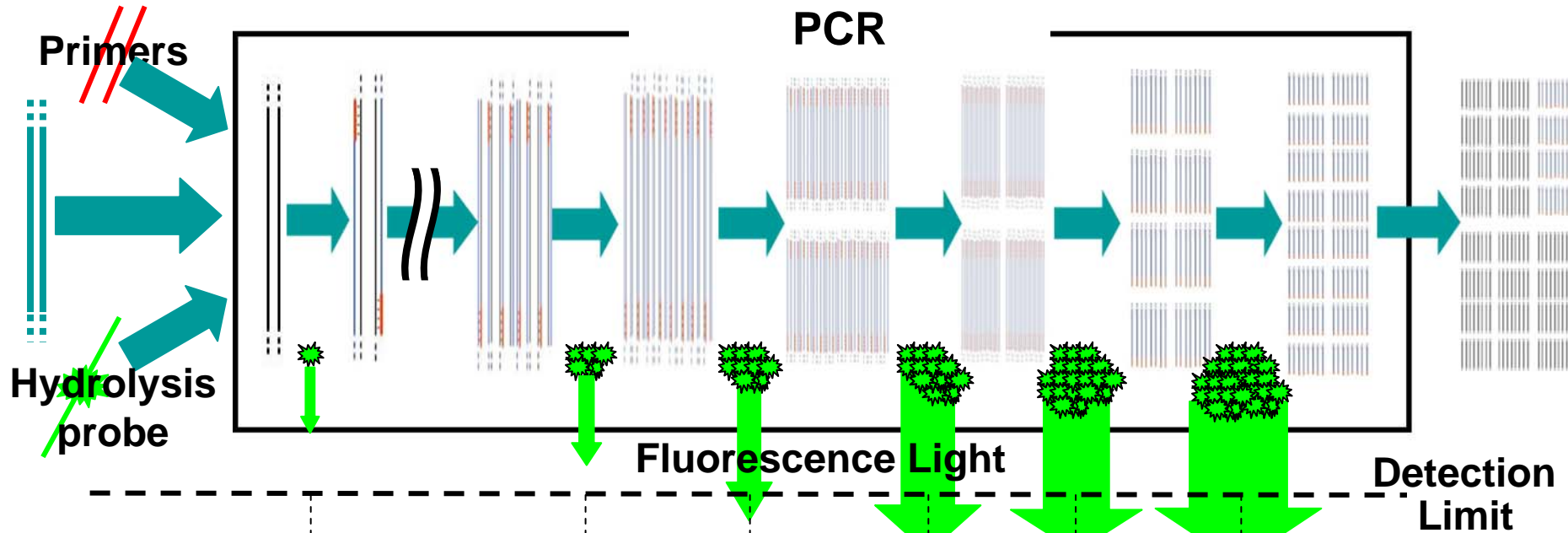
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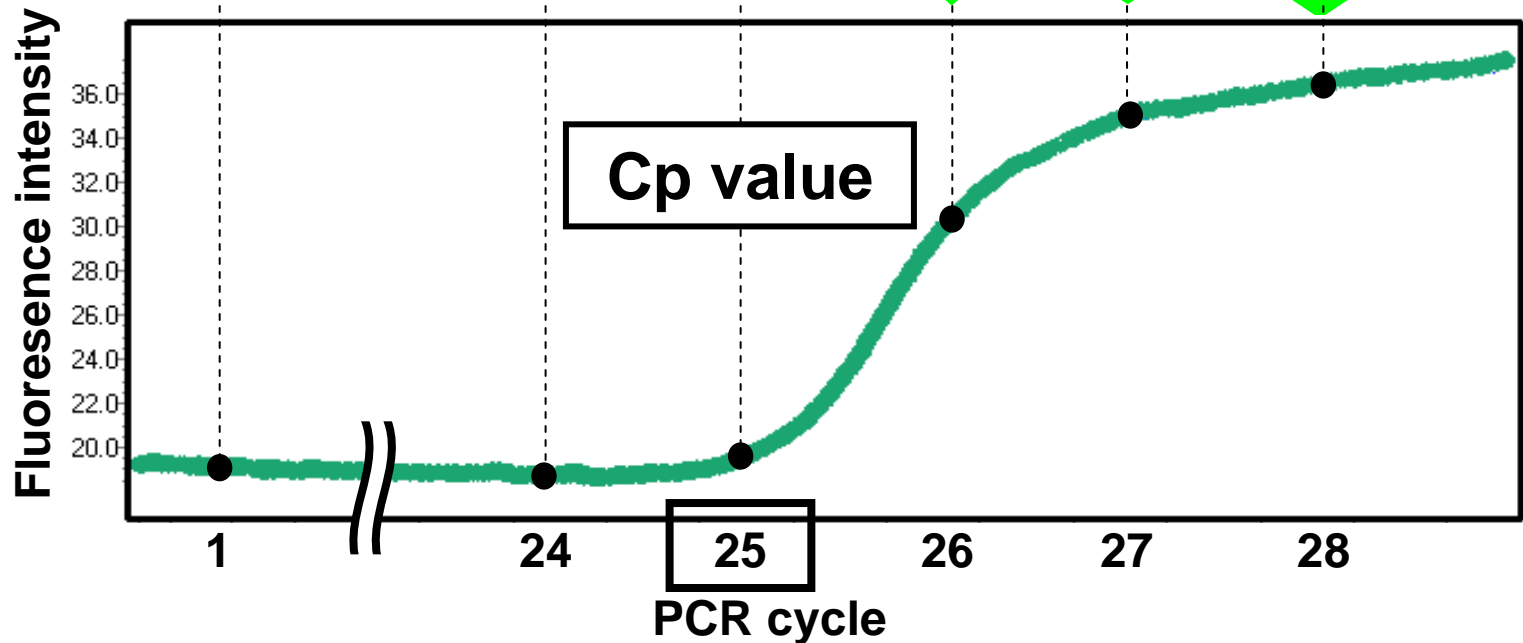
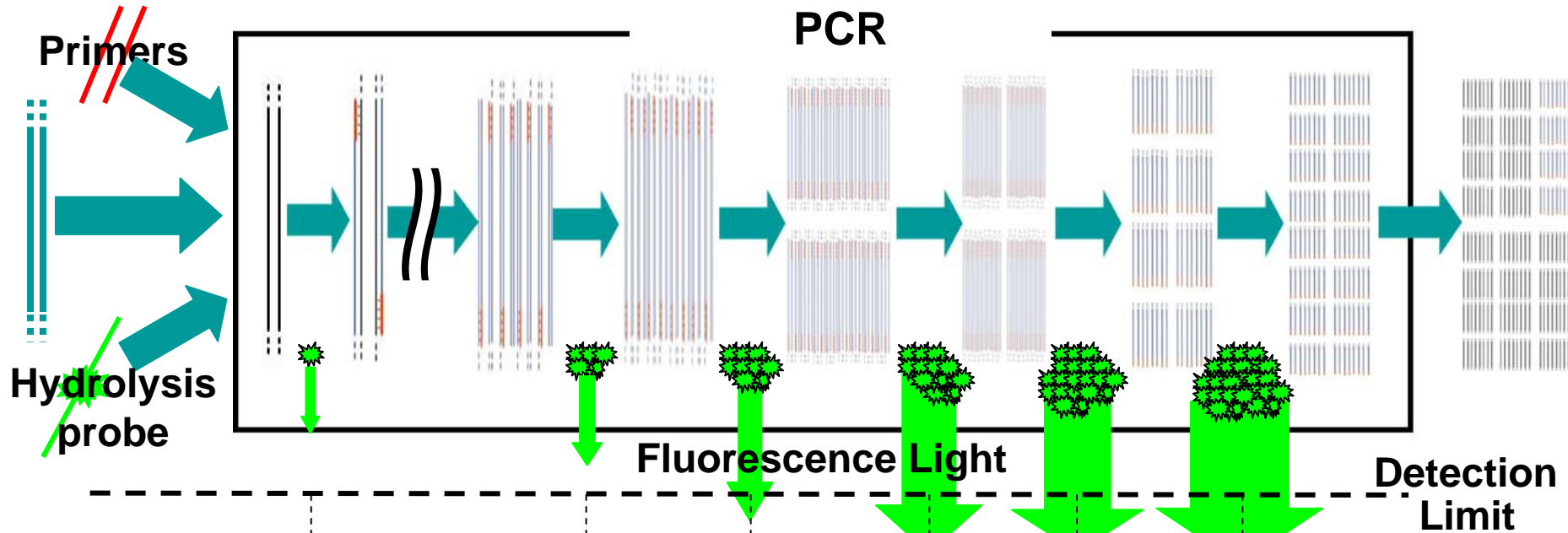
TaqMan probe Real-Time PCR method



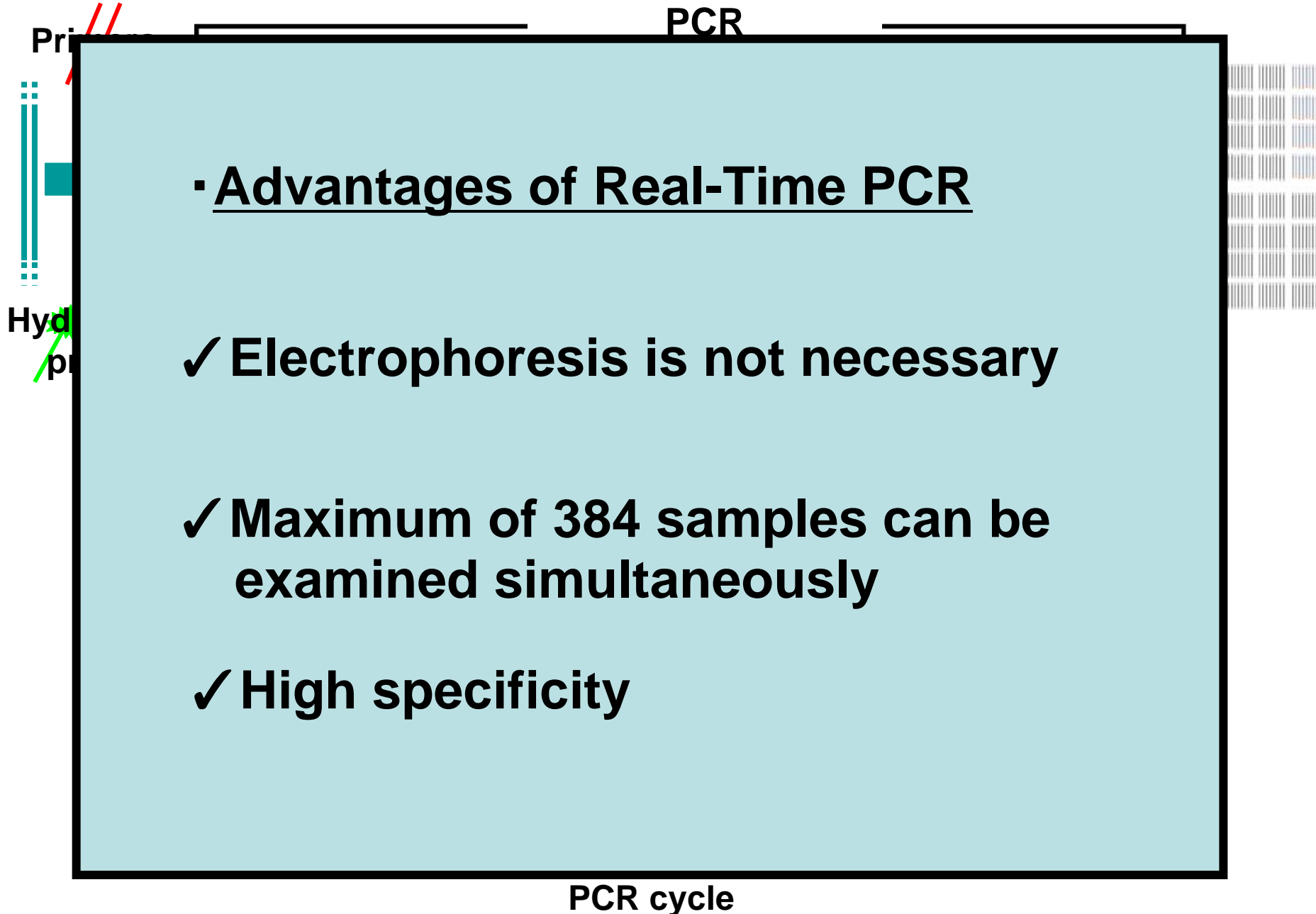
TaqMan probe Real-Time PCR method



TaqMan probe Real-Time PCR method



TaqMan probe Real-Time PCR method



Main Goals

Develop a molecular method for identify early life stages of *N. plumchrus* and *N. flemingeri* using Real-Time PCR

Investigate vertical distribution and seasonal migration of early life stages, and reveal early life history of *N. plumchrus* and *N. flemingeri*

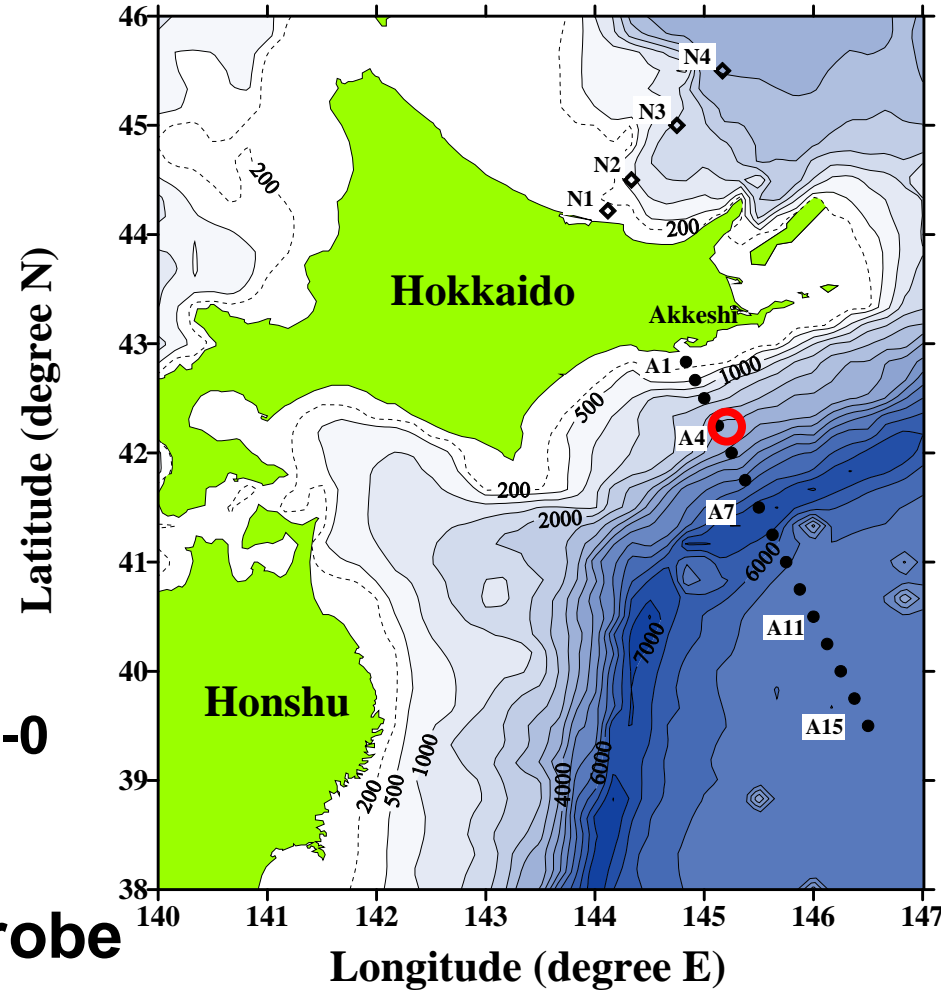
Materials and Methods

✓ Sampling

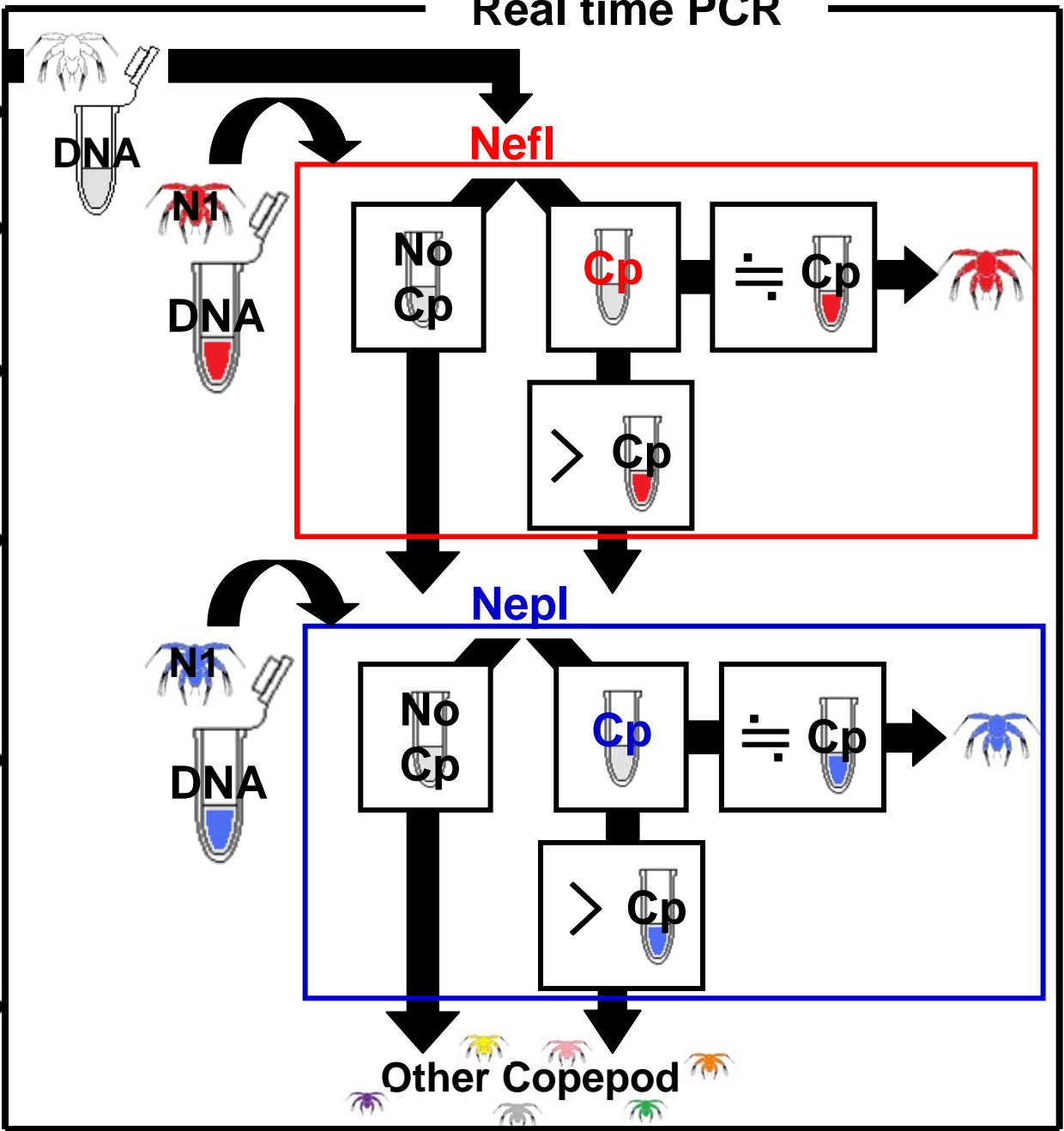
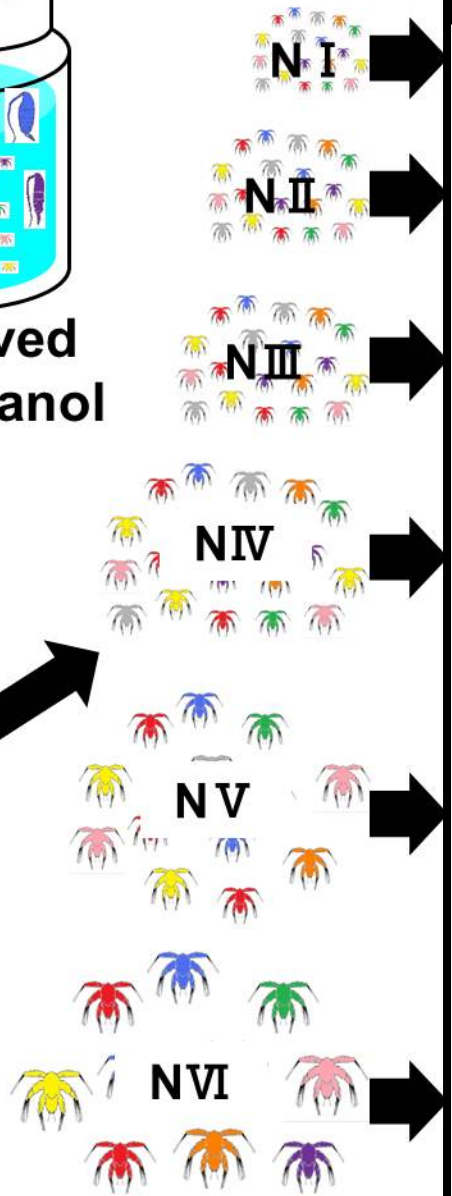
- Oyashio region A-line
- October 2009
January, March, April, May, June,
July 2010
- VMPS net and Closing Norpac net
2000-1500-1000-500-200-100-50-20-0

✓ Design of Primer pairs and Probe

- GenBank
Sequence data of Mitochondrial CO1 genes
N. plumchrus, *N. flemingeri*, *N. cristatus*
- 2 sets of specific primer pairs and probe : **Nefl** and **Nepl**

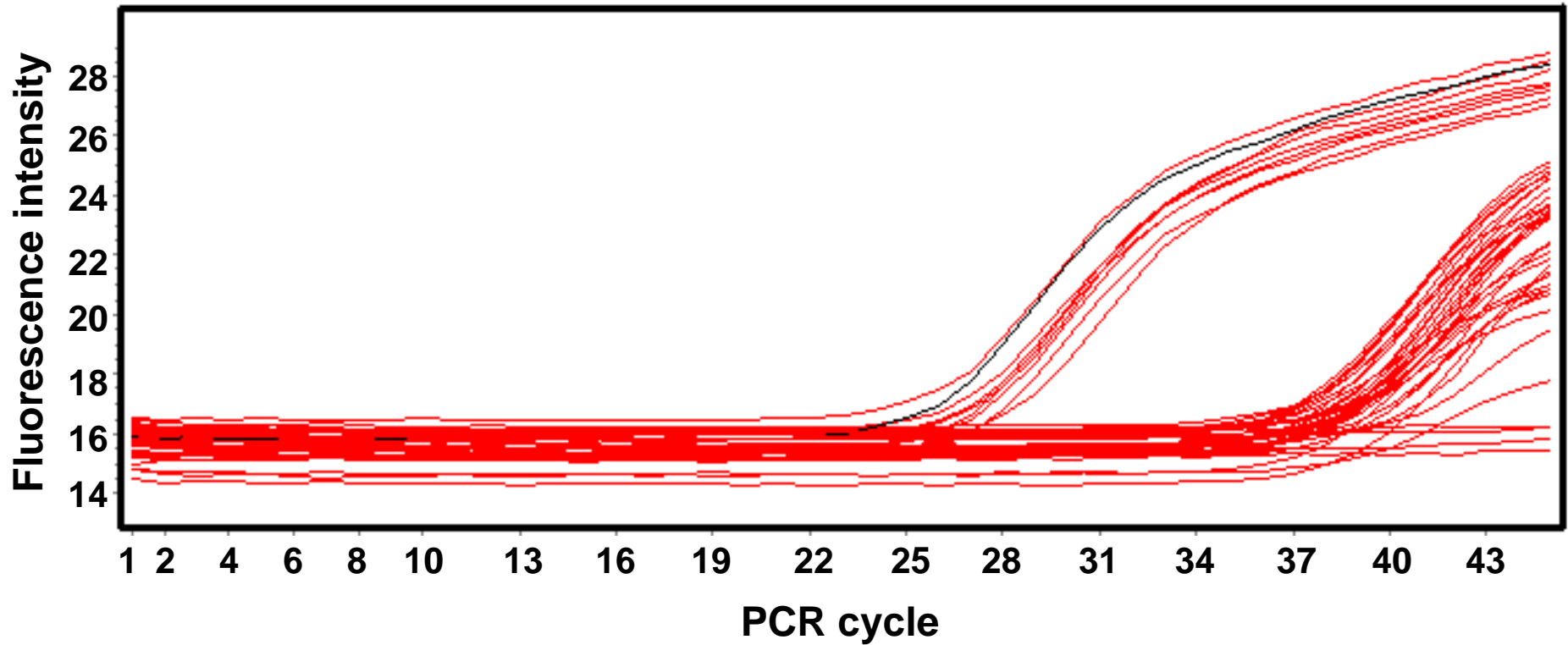


Procedure of identification



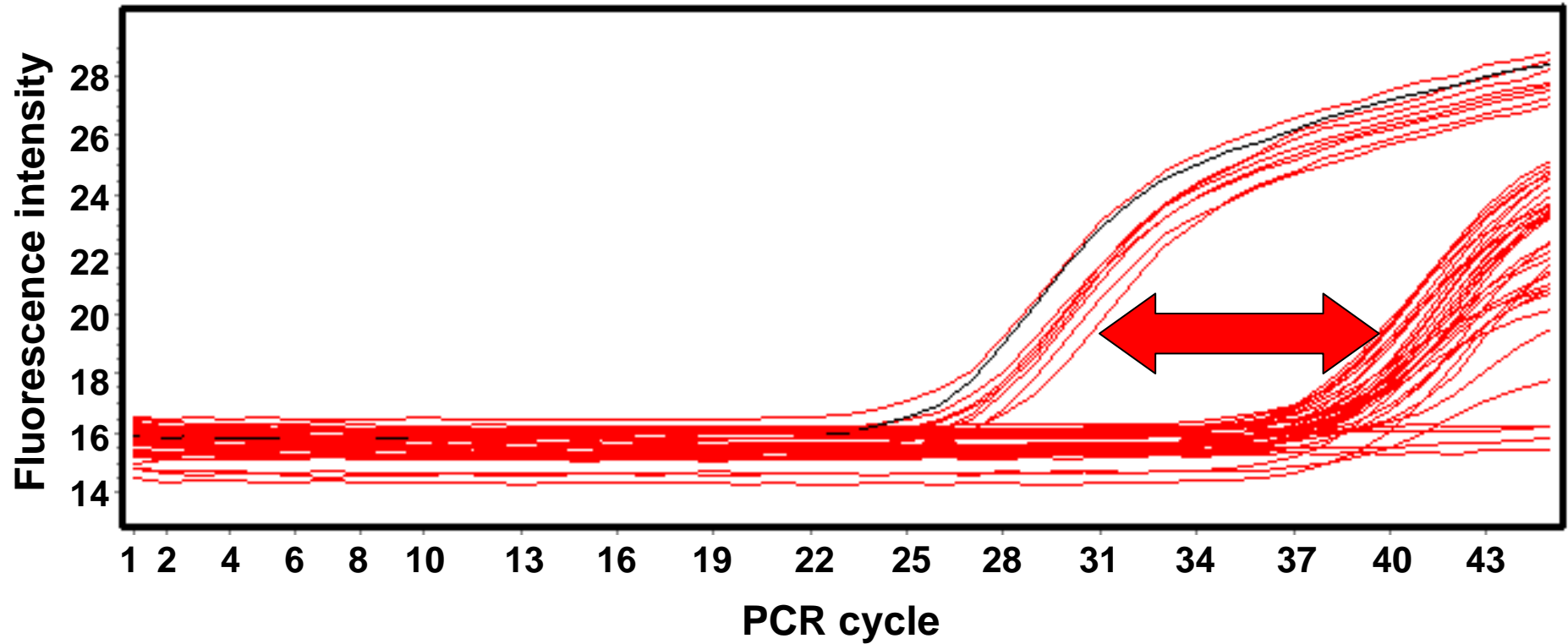
Result of Real-Time PCR

- Primer pairs and prob: **Nefl**
- Specimens: Stage 3 unknown nauplii from October 2009



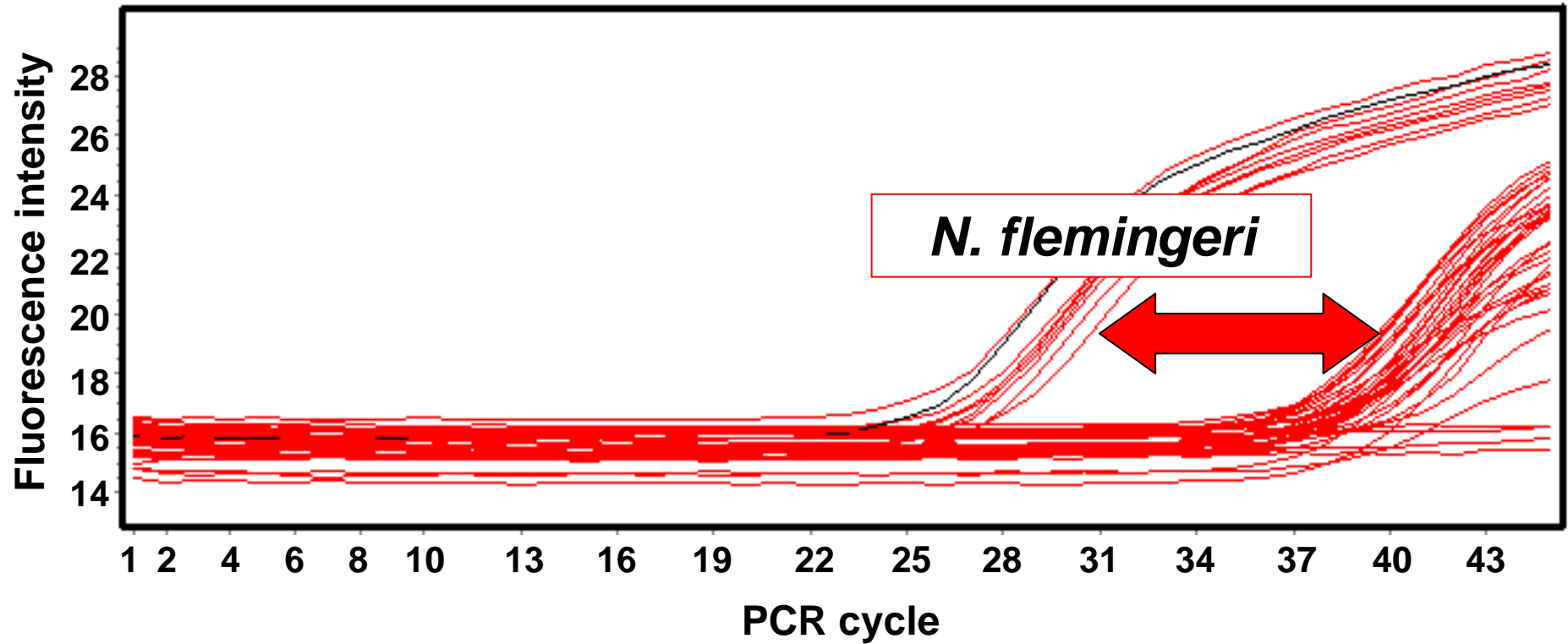
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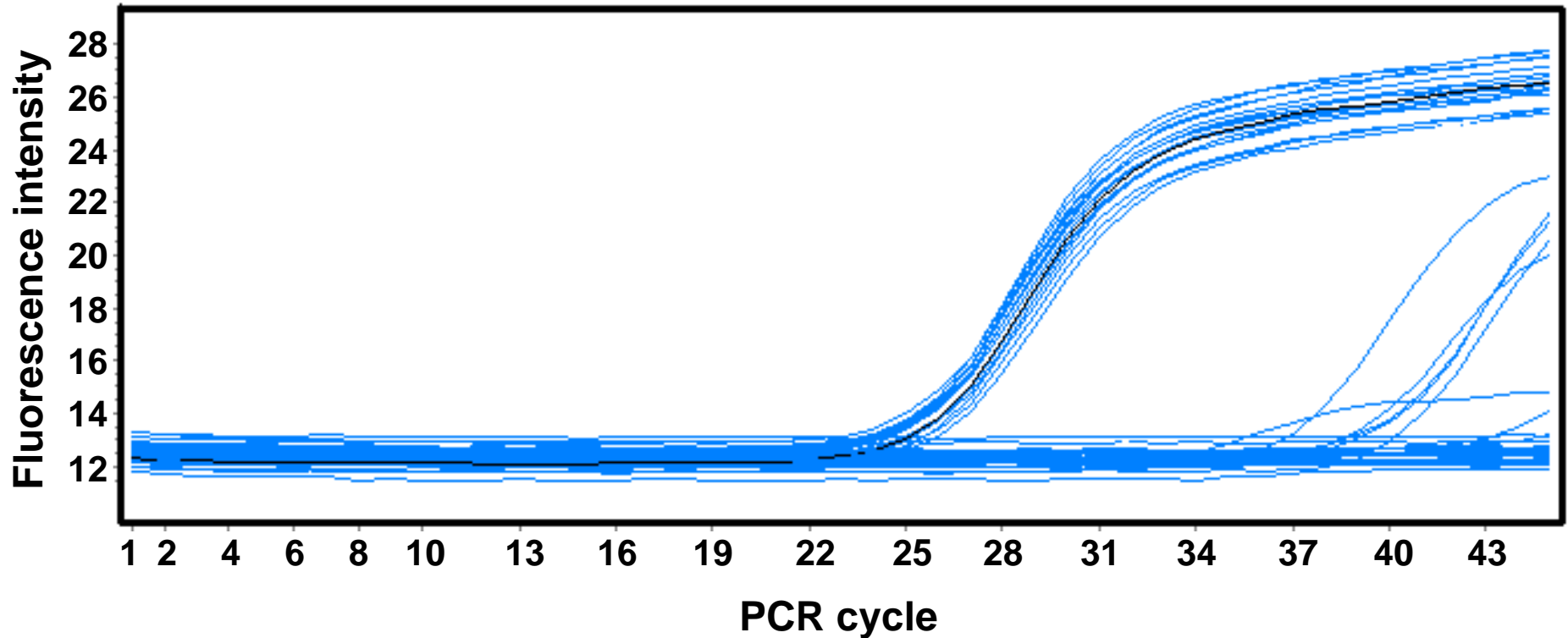
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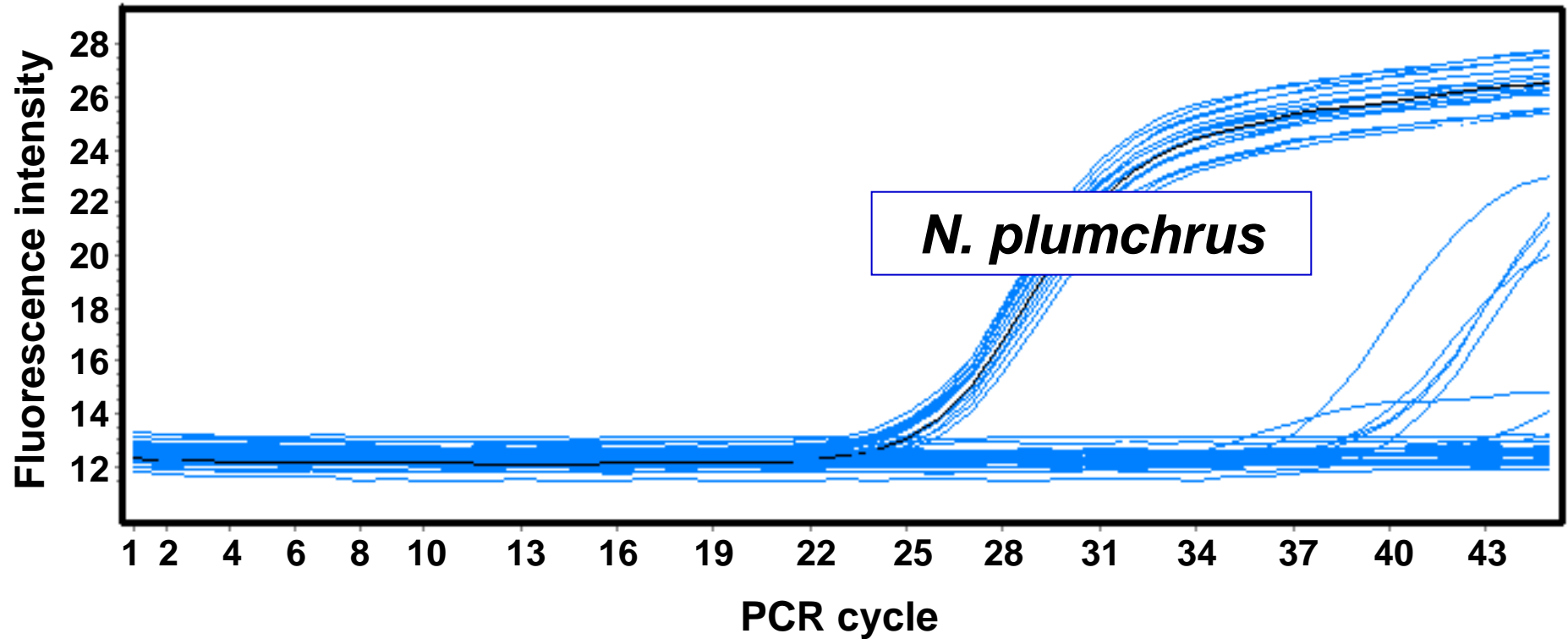
Result of Real-Time PCR

- Primer pairs and prob: **Nepl**
- Specimens: **Stage 3 unknown nauplii from October 2009**



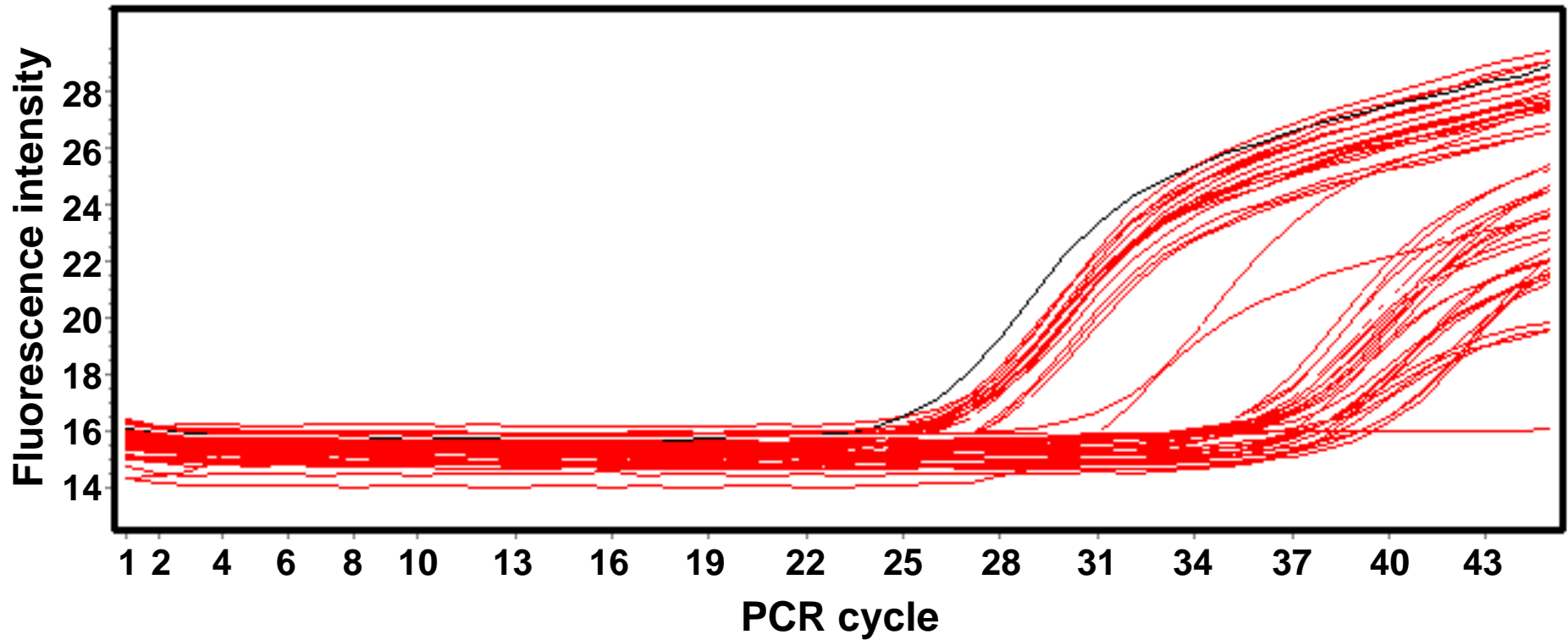
Result of Real-Time PCR

- Primer pairs and prob: **NepI**
- Specimens: Stage 3 unknown nauplii from October 2009



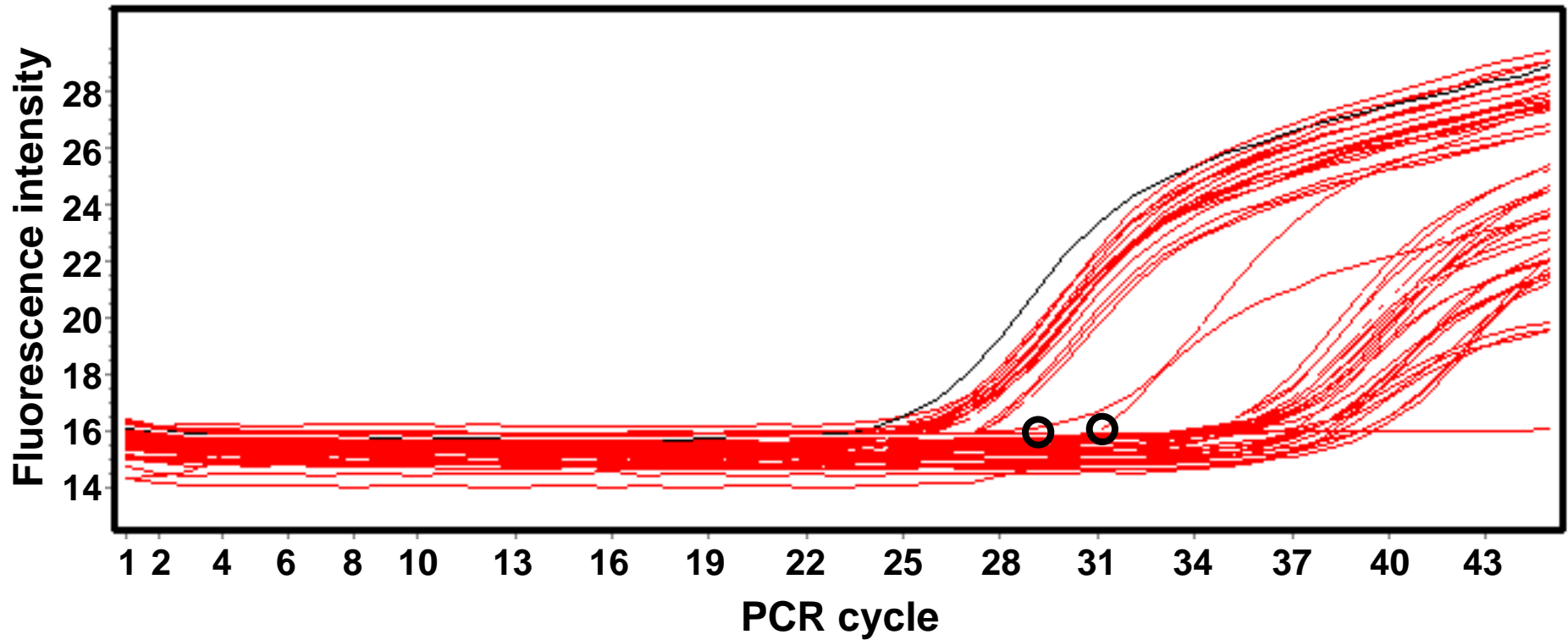
Result of Real-Time PCR

- Primer pairs and prob: **Nefl**
- Specimens: Stage 3 unknown nauplii from January 2010



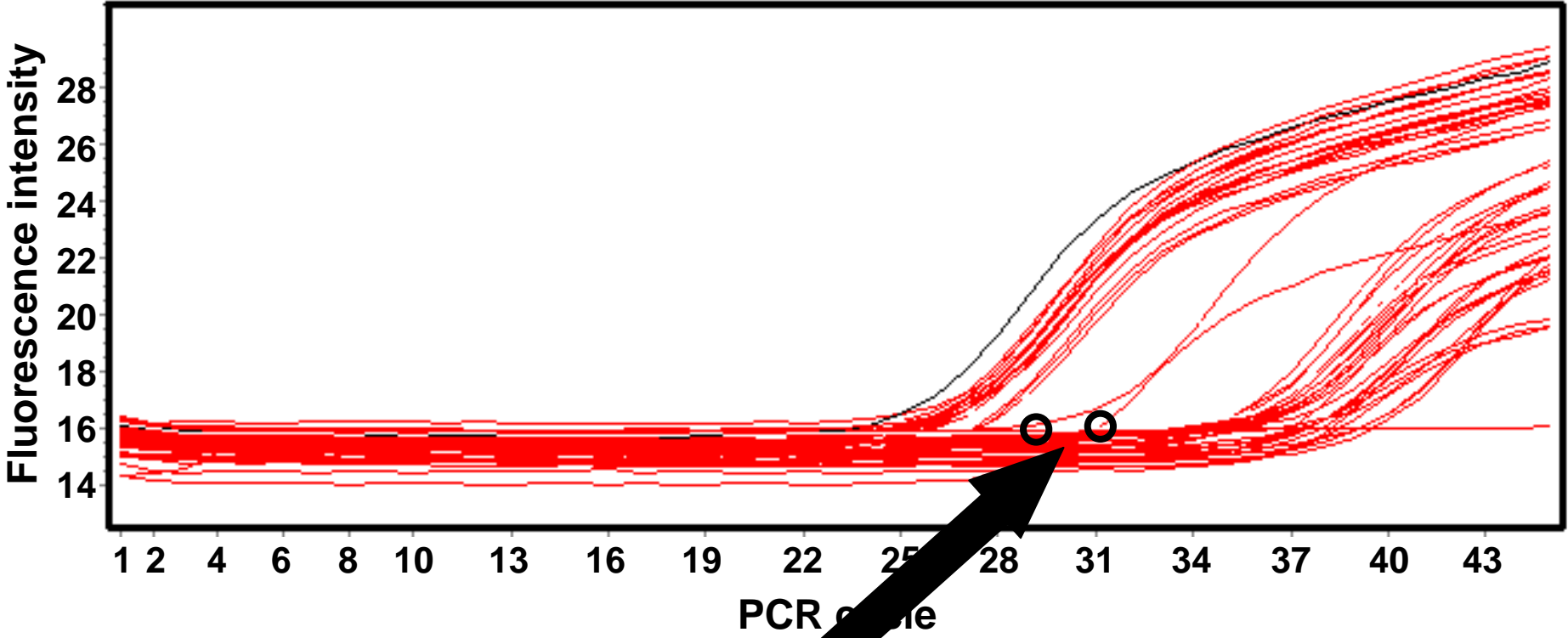
Result of Real-Time PCR

- Primer pairs and prob: **Nefl**
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Result of Real-Time PCR

- Primer pairs and prob: **Nefl**
- Specimens: Stage 3 unknown nauplii from January 2010



Unable to distinguish

**Number of
undistinguishable
nauplii**

**Number of
nauplii**

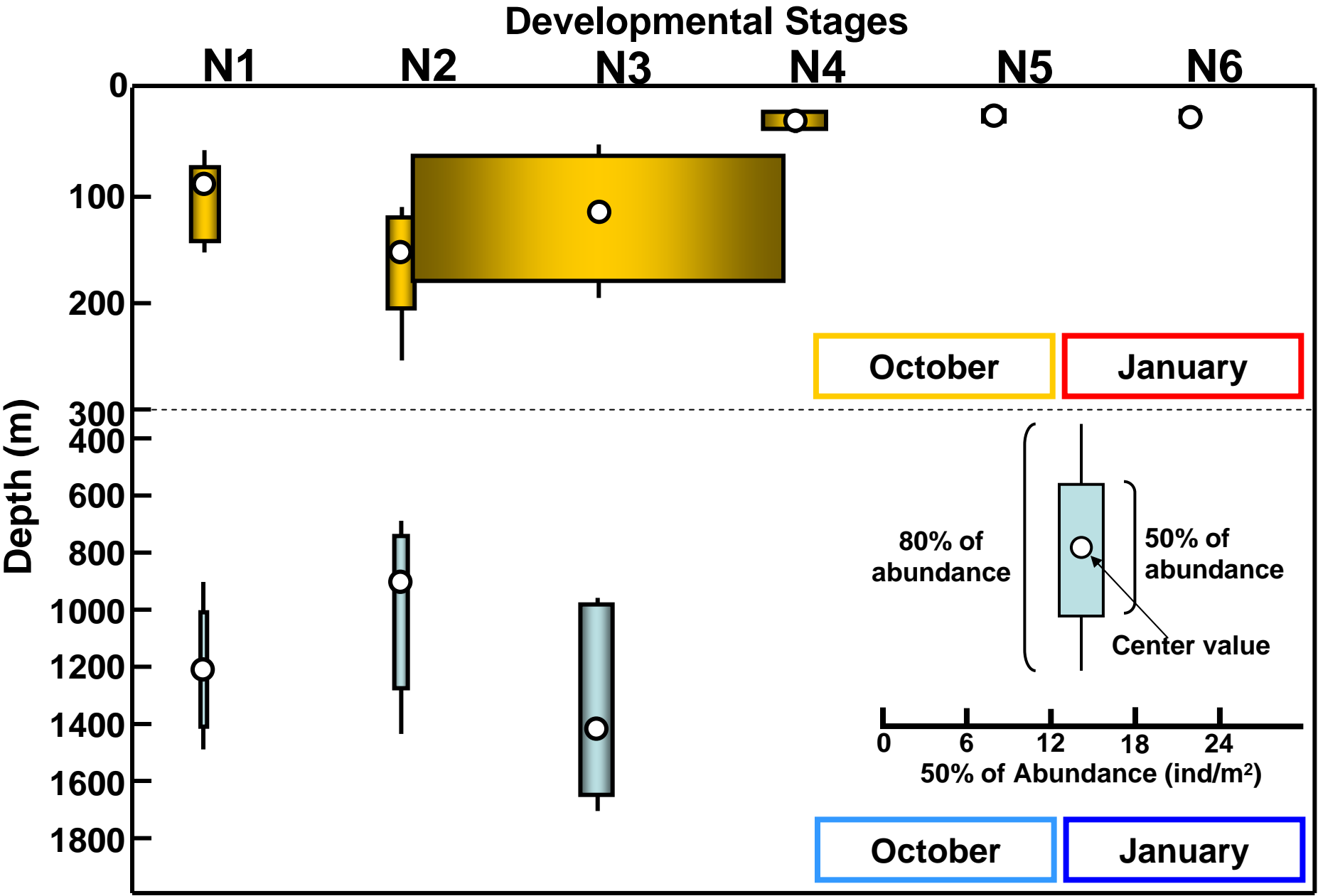
=

2580

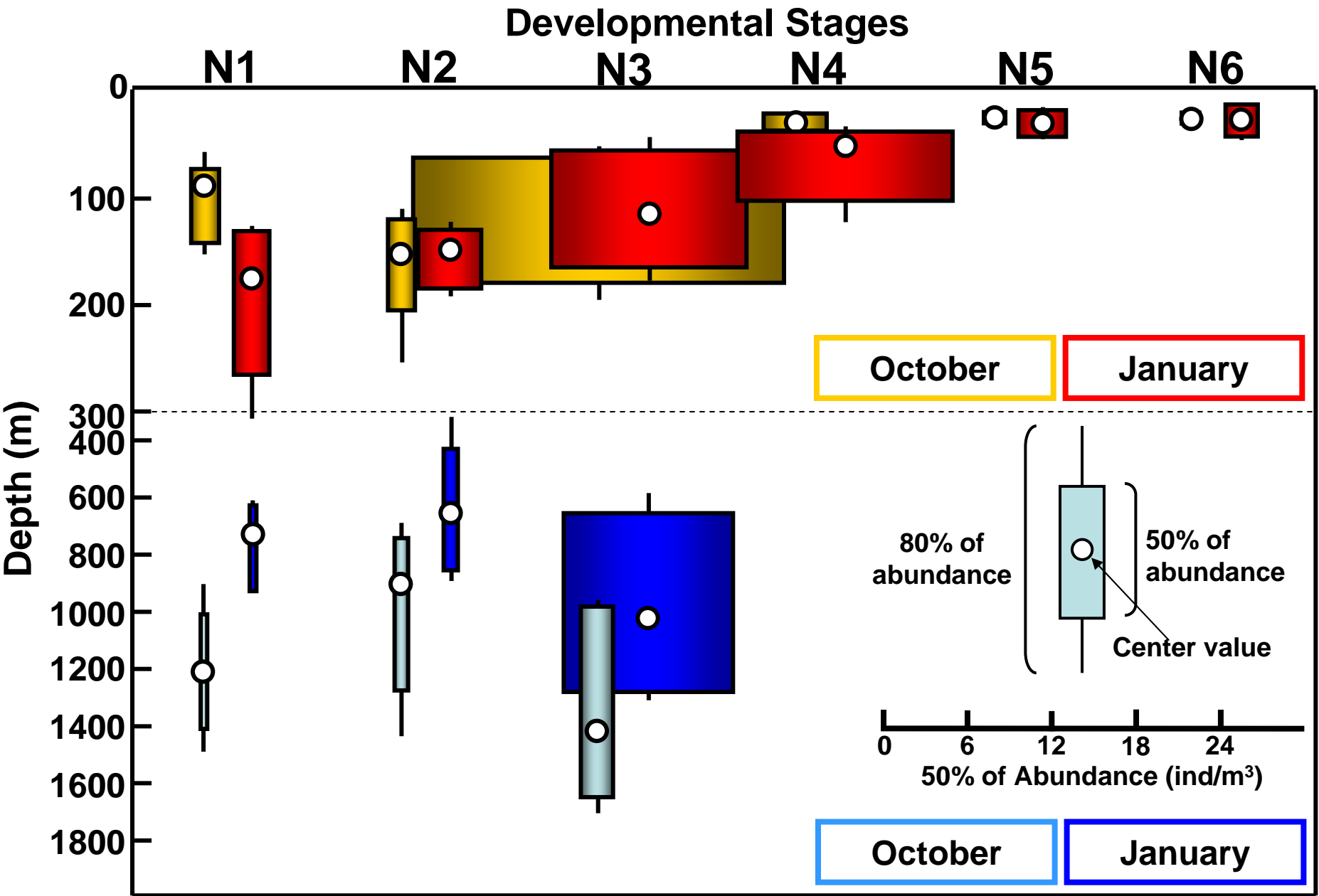
$$\frac{\text{Number of undistinguishable nauplii}}{\text{Number of nauplii}} = \frac{19}{2580}$$

- **0.7% of specimens are undistinguishable**
- **Real-Time PCR method can be effective tool for identify early life stage of *Neocalanus***

Vertical distribution of *N. plumchrus* & *N. flemingeri*



Vertical distribution of *N. plumchrus* & *N. flemingeri*



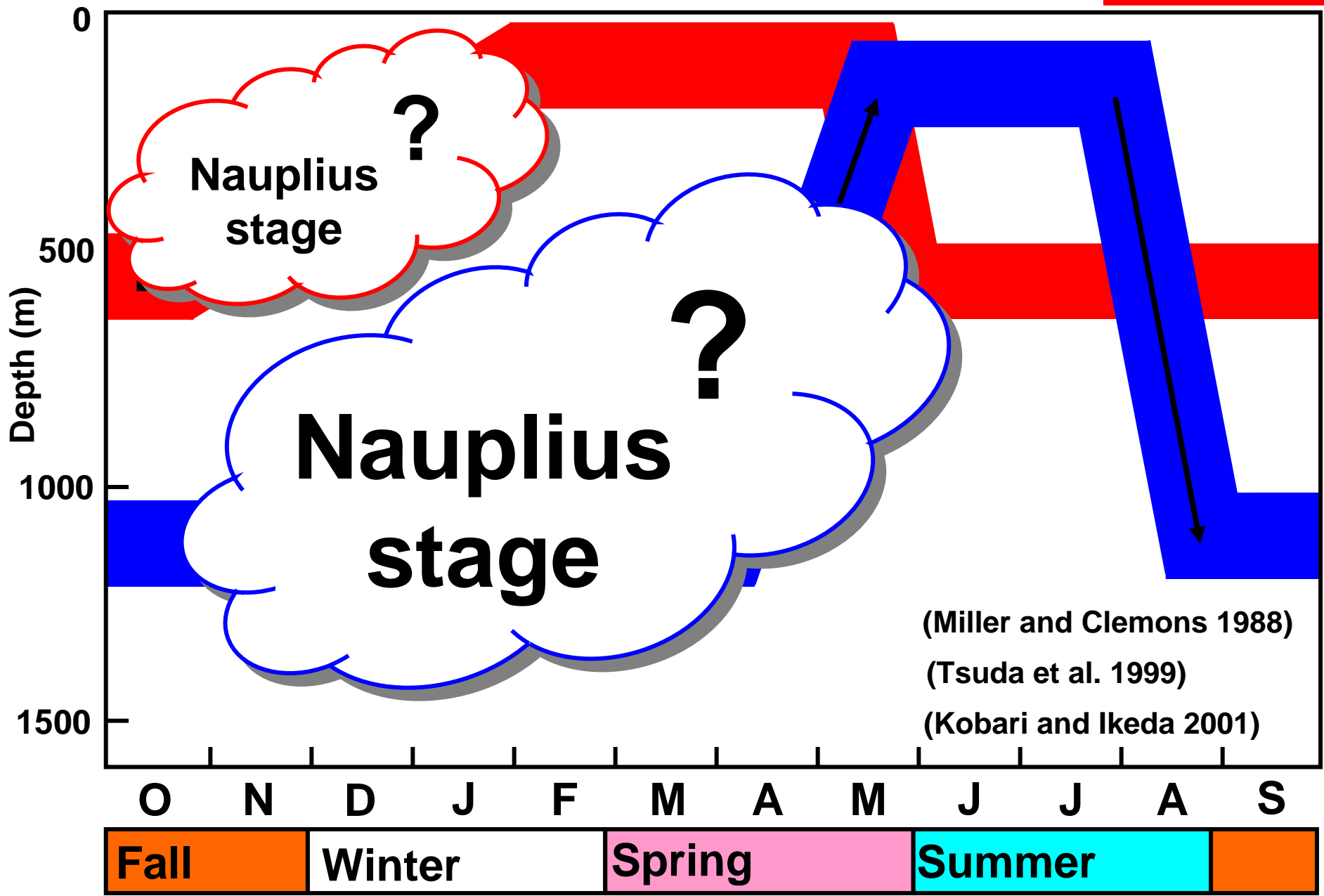
Discussion & Conclusion

✓ Real-Time PCR method

- **0.7% of specimens are undistinguishable**
- **Effective tool for identify nauplii of *Neocalanus***
- **Results revealed imagination part of life history of *N. plumchrus* and *N. flemingeri***

Vertical distribution of nauplius stage

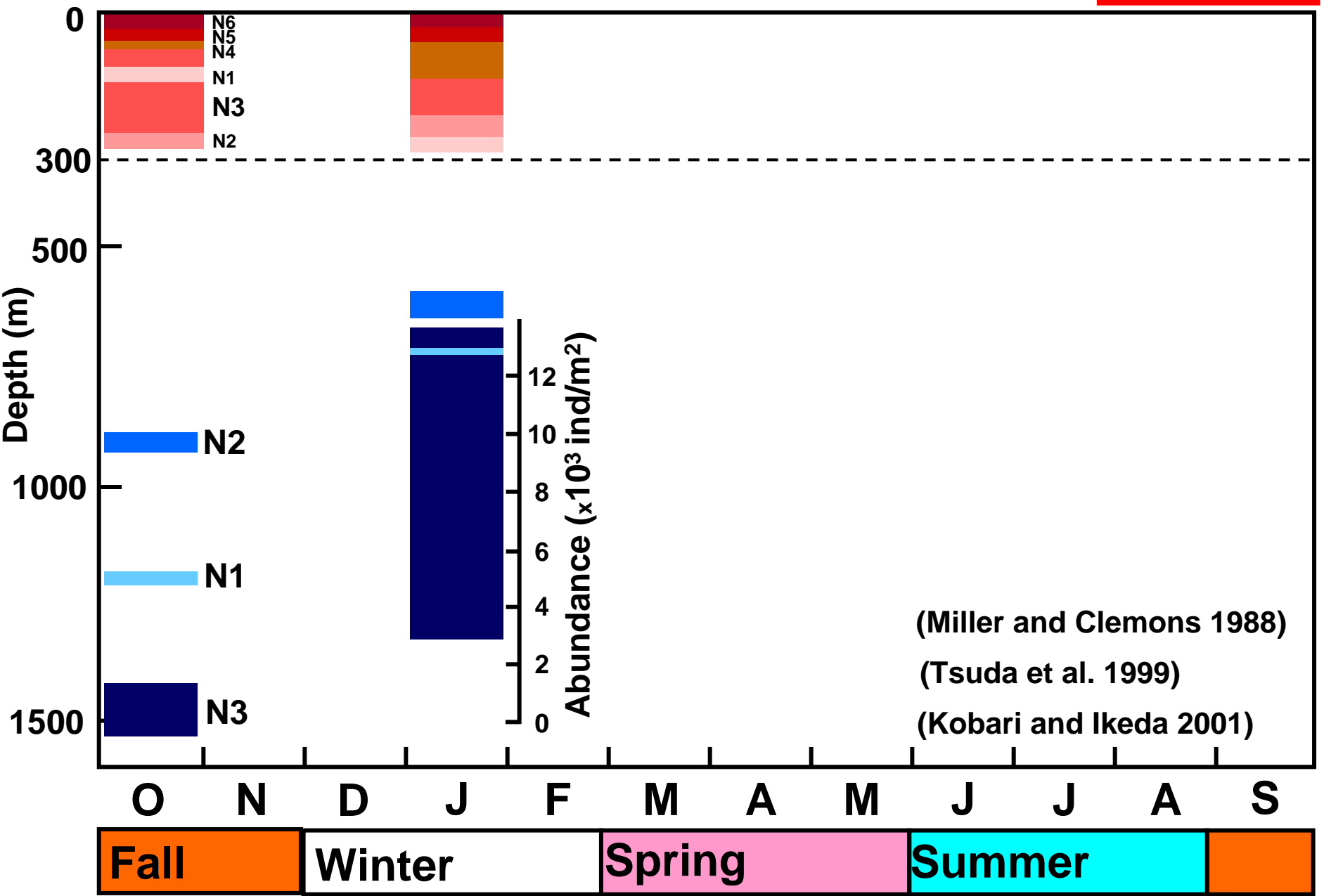
N. plumchrus
N. flemingeri



(Miller and Clemons 1988)
(Tsuda et al. 1999)
(Kobari and Ikeda 2001)

Vertical distribution of nauplius stage

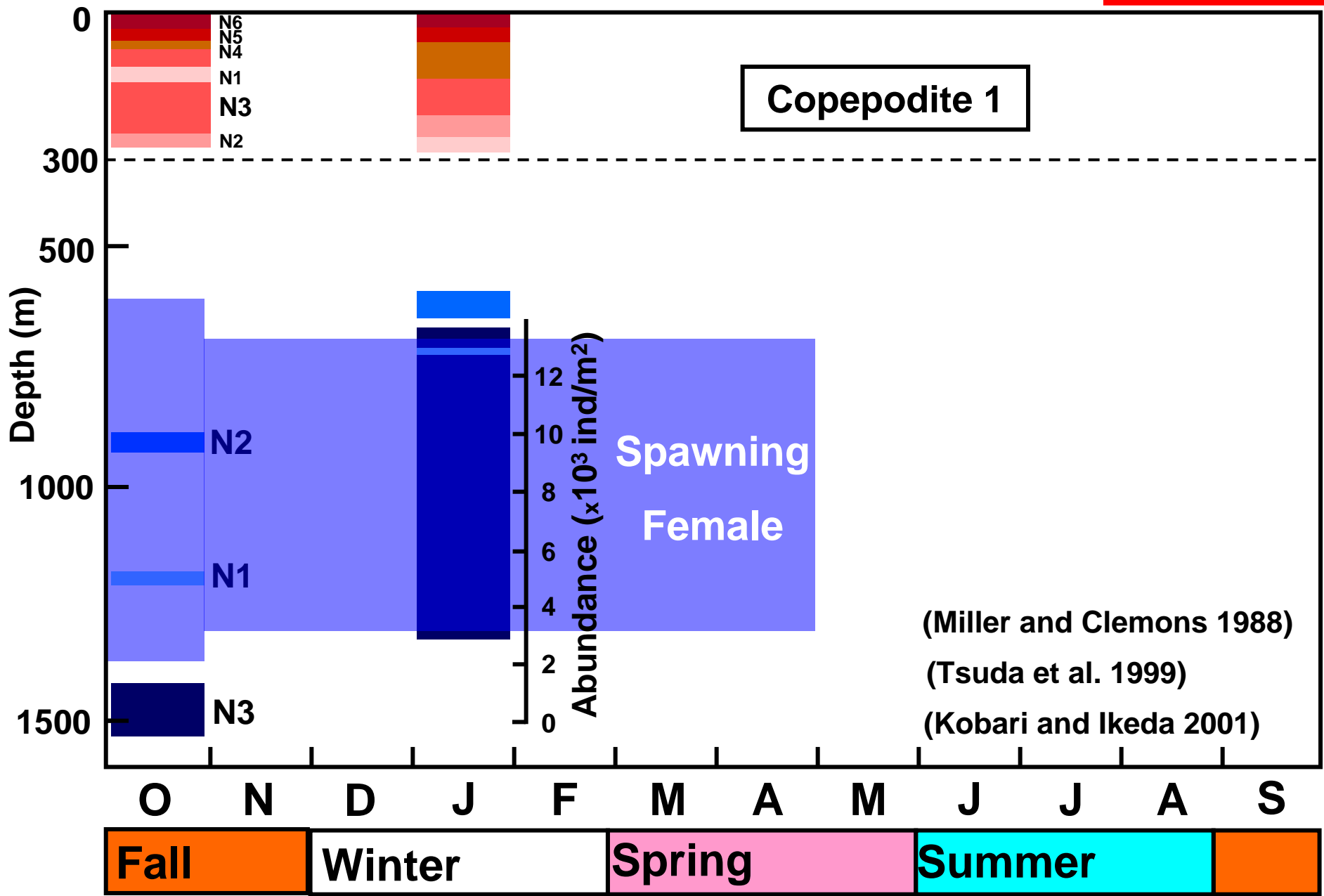
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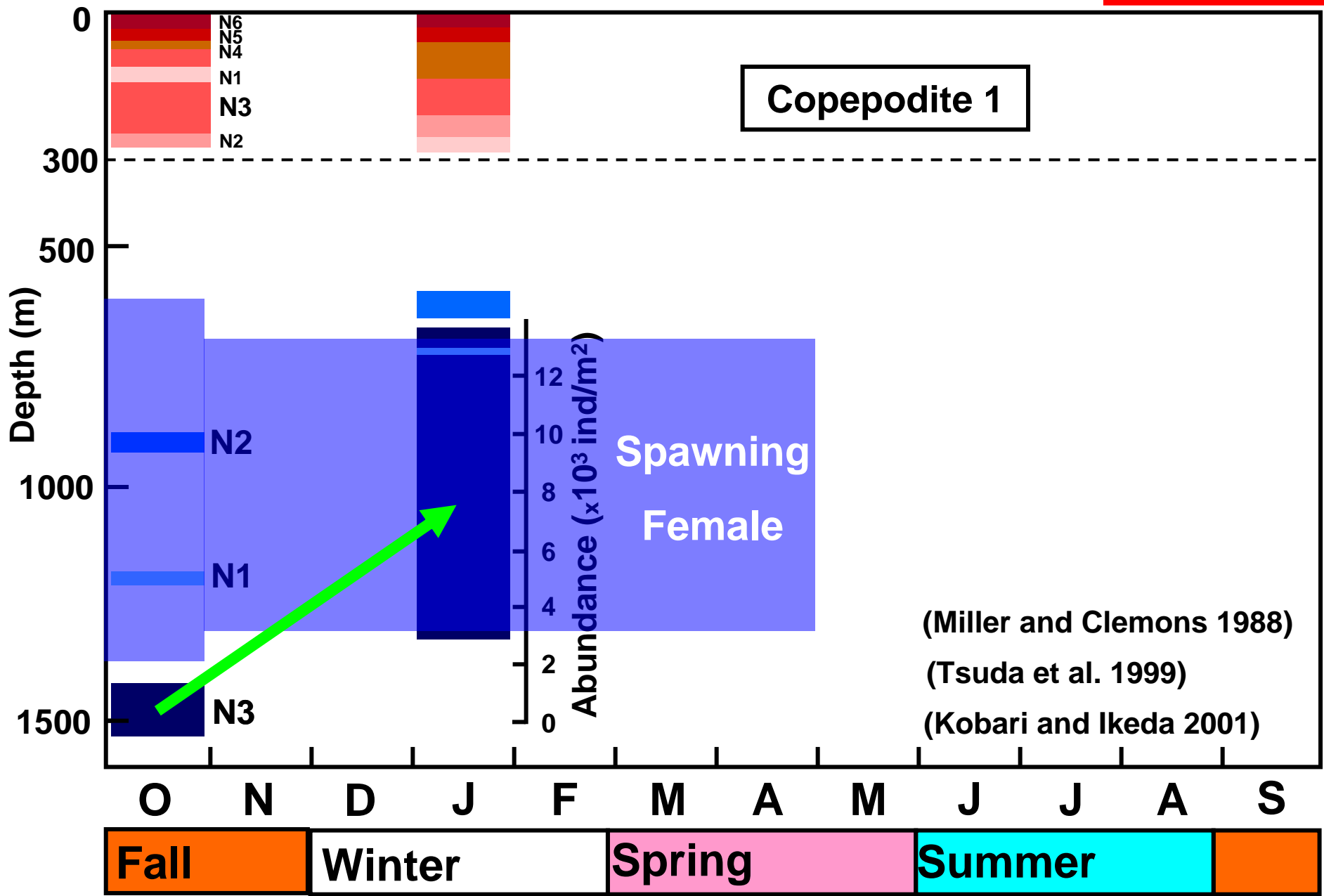
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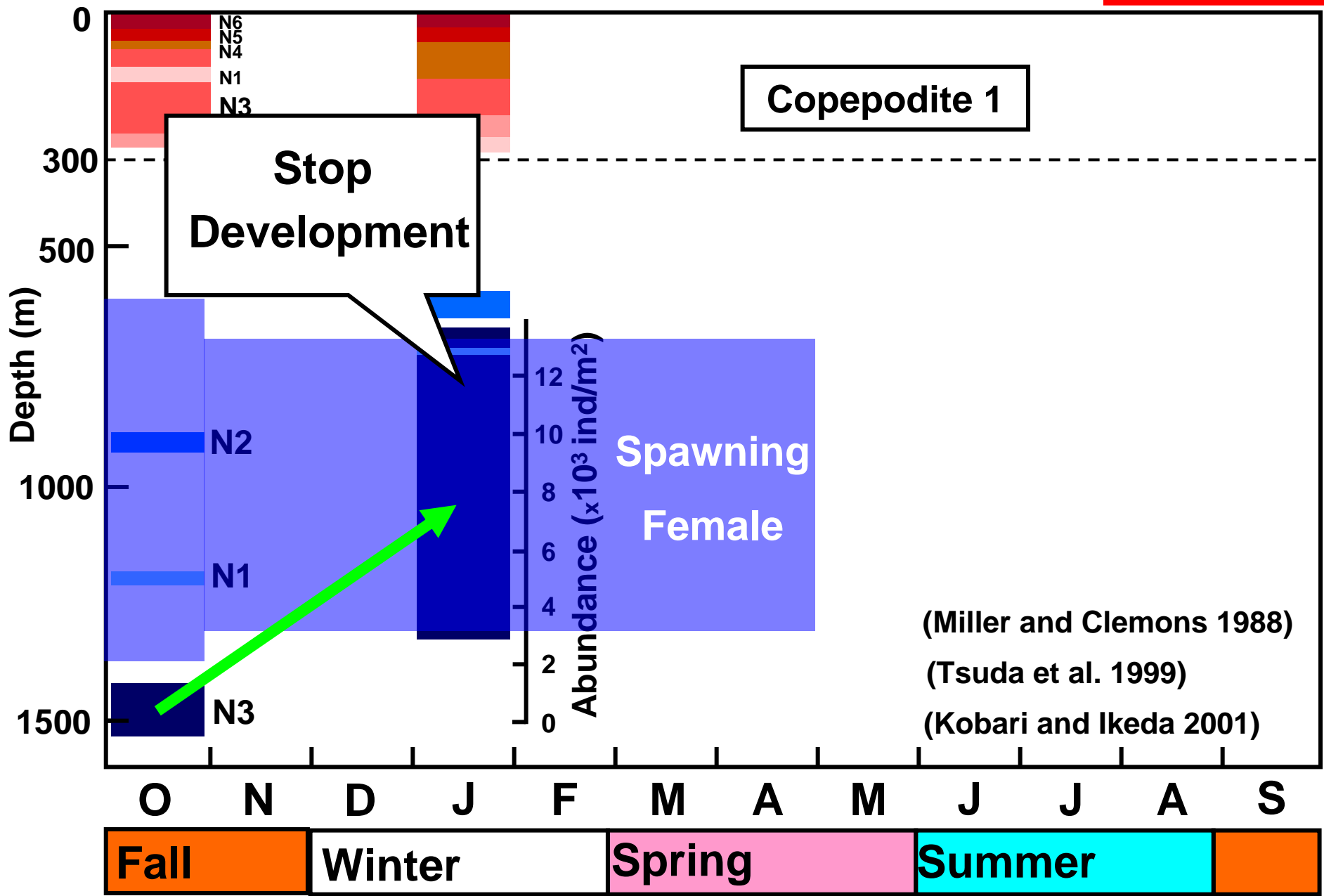
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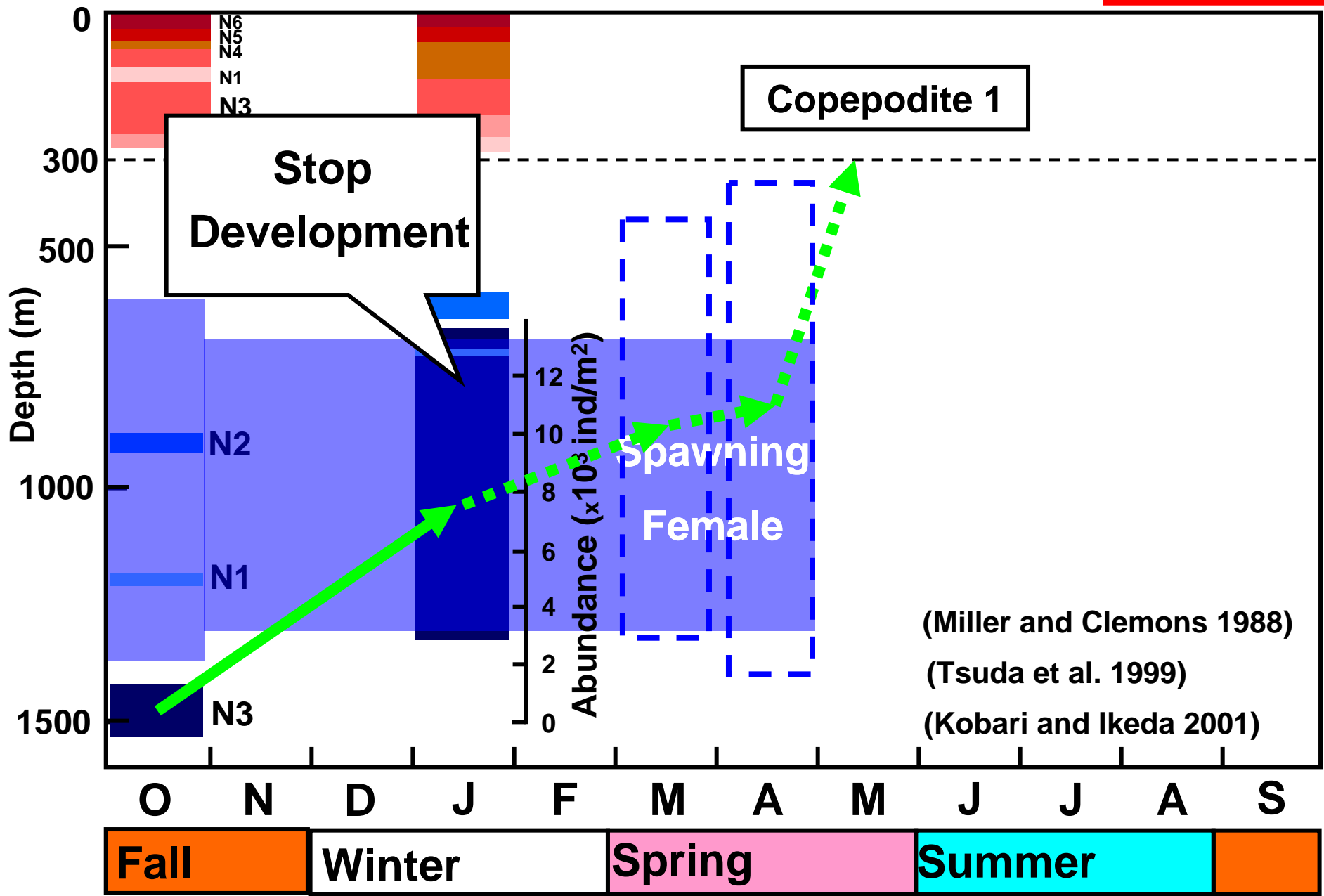
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